

(12) PATENT

(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199856180 B2

(10) Patent No. 713471

(54) Title

Ligand for receptor activator of NF-kappa B, ligand is member of TNF superfamily

(51) International Patent Classification(s)

C12N 015/15 C07K 016/28
C07K 014/52 C12N 015/11
C07K 014/705 C12N 015/62
C07K 016/24

(21) Application No: 199856180

(22) Application Date: 1997.12.22

(87) WIPO No: WO98/28426

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/059978	1996.12.23	US
60/011509	1997.03.07	US
60/044671	1997.10.14	US

(43) Publication Date : 1998.07.17

(43) Publication Journal Date : 1998.09.10

(44) Accepted Journal Date : 1999.12.02

(71) Applicant(s)

Immunex Corporation

(72) Inventor(s)

Dirk M. Anderson; Laurent J. Galibert; Eugene Marakovskiy

(74) Agent/Attorney

PHILLIPS ORMONDE and FITZPATRICK, 367 Collins Street, MELBOURNE VIC 3000

BEST AVAILABLE COPY

**CORRECTED
VERSION***

PCT

page 1 and 2, description, replaced by new pages 1 and 2; page 2/6,
drawings, replaced by a new page 2/6; due to late transmission by the
receiving Office

56180/18

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5:	C12N 15/19, 15/62, C07K 16/24, 16/28 C12N 15/11, C07K 14/52, 14/705		
A3	(11) International Publication Number: WO 98/28426		
	(43) International Publication Date: 2 July 1998 (02.07.98)		
(21) International Application Number: PCT/US97/27715		(81) Designated States: AL, AM, AU, BA, BB, BO, BR, CA, CN, CU, CZ, DK, EE, GE, IL, IS, JP, KP, KR, LC, LK, LR, LT, LY, MN, MD, MR, NO, PL, RO, SE, SI, SK, SL, TM, TT, UA, UZ, VN, YU, ARDPA (AM, AZ, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AT, BE, BY, KG, KZ, MD, RU, TI, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 22 December 1997 (22.12.97)			
(30) Priority Data: 60/059,971 23 December 1996 (23.12.96) US 60/013,509 7 March 1997 (07.03.97) US 60/064,671 14 October 1997 (14.10.97) US			
(71) Applicant: IMMUNEX CORPORATION (US/US); Law Dept., 51 University Street, Seattle, WA 98101 (US).		Published With international search report.	
(72) Inventor: ANDERSSON, Dick, M.; 3616 NW 64th Street, Seattle, WA 98107 (US). GALIBERT, Laurent, J.; 617 5th Avenue West, Seattle, WA 98119 (US). MARASCOVSKY, Eugene; 4123 Evanston Avenue North, Seattle, WA 98103 (US).		(85) Date of publication of the international search report: 1 October 1998 (01.10.98)	
(74) Agent: PERKINS, Patricia, Anne; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).			
(54) Title: LIGAND FOR RECEPTOR ACTIVATOR OF NF-KAPPA B, LIGAND IS MEMBER OF TNF SUPERFAMILY			
(57) Abstract			
Isolated ligands, DNAs encoding such ligands, and pharmaceutical compositions made therefrom, are disclosed. The isolated ligands can be used to regulate an immune response. The ligands are also useful in screening for inhibitors thereof.			
IP AUSTRALIA 1 FEB 1998			

*(Referred to in PCT Gazette No. 3/1999, Section II)

TITLE

LIGAND FOR RECEPTOR ACTIVATOR OF NF-KAPPA B, LIGAND IS MEMBER OF TNF SUPERFAMILY

5

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokines, and more specifically to cytokine receptor/ligand pairs having immunoregulatory activity.

BACKGROUND OF THE INVENTION

Efficient functioning of the immune system requires a fine balance between cell proliferation and differentiation and cell death, to ensure that the immune system is capable of reacting to foreign, but not self antigens. Integral to the process of regulating the immune and inflammatory response are various members of the Tumor Necrosis Factor (TNF) Receptor/Nerve Growth Factor Receptor superfamily (Smith et al., *Science* 248:1019, 1990). This family of receptors includes two different TNF receptors (Type I and Type II; Smith et al., *supra*; and Schall et al., *Cell* 61:361, 1990), nerve growth factor receptor (Johnson et al., *Cell* 47:545, 1986), B cell antigen CD40 (Stamenkovic et al., *EMBO J.* 8:1403, 1989), CD27 (Camerini et al., *J. Immunol.* 147:3165, 1991), CD30 (Durkop et al., *Cell* 68:421, 1992), T cell antigen OX40 (Mallet et al., *EMBO J.* 9: 1063, 1990), human *Fas* antigen (Itoh et al., *Cell* 66:233, 1991), murine 4-1BB receptor (Kwon et al., *Proc. Natl. Acad. Sci. USA* 86:1963, 1989) and a receptor referred to as Apoptosis-Inducing Receptor (AIR; USSN 08/720,864, filed October 4, 1996).

CD40 is a receptor present on B lymphocytes, epithelial cells and some carcinoma cell lines that interacts with a ligand found on activated T cells, CD40L (USSN 08/249,189, filed May 24, 1994). The interaction of this ligand/receptor pair is essential for both the cellular and humoral immune response. Signal transduction via CD40 is mediated through the association of the cytoplasmic domain of this molecule with members of the TNF receptor-associated factors (TRAFs; Baker and Reddy, *Oncogene* 12:1, 1996). It has recently been found that mice that are defective in TRAF3 expression due to a targeted disruption in the gene encoding TRAF3 appear normal at birth but develop progressive hypoglycemia and depletion of peripheral white cells, and die by about ten days of age (Xu et al., *Immunity* 5:407, 1996). The immune responses of chimeric mice reconstituted with TRAF3^{-/-} fetal liver cells resemble those of CD40-deficient mice, although TRAF3^{-/-} B cells appear to be functionally normal.

The critical role of TRAF3 in signal transduction may be in its interaction with one of the other members of the TNF receptor superfamily, for example, CD30 or CD27.

which are present on T cells. Alternatively, there may be other, as yet unidentified members of this family of receptors that interact with TRAF3 and play an important role in postnatal development as well as in the development of a competent immune system. Identifying additional members of the TNF receptor superfamily would provide an additional means of regulating the immune and inflammatory response, as well as potentially providing further insight into post-natal development in mammals.

SUMMARY OF THE INVENTION

The present invention provides a counterstructure, or ligand, for a novel receptor referred to as RANK (for receptor activator of NF- κ B), that is a member of the TNF superfamily. The ligand, which is referred to as RANKL, is a Type 2 transmembrane protein with an intracellular domain of less than about 50 amino acids, a transmembrane domain and an extracellular domain of from about 240 to 250 amino acids. Similar to other members of the TNF family to which it belongs, RANKL has a 'spacer' region between the transmembrane domain and the receptor binding domain that is not necessary for receptor binding. Accordingly, soluble forms of RANKL can comprise the entire extracellular domain or fragments thereof that include the receptor binding region.

RANK is a Type 1 transmembrane protein having 616 amino acid residues that is a member of the TNFR superfamily, and interacts with TRAF3. Triggering of RANK by over-expression, co-expression of RANK and membrane bound RANKL, or by soluble RANKL or agonistic antibodies to RANK, results in the upregulation of the transcription factor NF- κ B, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the influence of RANK.Fc and hRANKL on activated T cell growth. Human peripheral blood T cells were cultured as described in Example 12; viable 30 T cell recovery was determined by triplicate trypan blue counting.

Figure 2 illustrates the ability of RANKL to induce human DC cluster formation. Functionally mature dendritic cells (DC) were generated *in vitro* from CD34 $^{+}$ bone marrow (BM) progenitors and cultured as described in Example 13. CD11 α $^{+}$ DC were cultured in a cytokine cocktail alone (Figure 2A), in cocktail plus CD40L (Figure 2B), RANKL (Figure 35 2C), or heat inactivated (AH) RANKL (Figure 2D), and then photographed using an inversion microscope.

Figure 3 demonstrates that RANKL enhances DC allo-stimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as

described in Example 13. The cultures were pulsed with [³H]-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the mean \pm standard deviation (SD) of triplicate cultures.

- Figure 4 presents an alignment of human RANK with other TNFR family members
5 in the region of structurally conserved extracellular cysteine-rich pseudorepeats. Predicted disulfide linkages (DS1-DS3) are indicated. RANK and CD40 contain identical amino acid substitutions (C^H, C^G) eliminating DS2 in the second pseudorepeat.

Figure 5 presents an alignment of human RANKL with other TNF family members.

10 **DETAILED DESCRIPTION OF THE INVENTION**

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was used to hybridize to colony blots generated from a DC cDNA library containing full-length cDNAs. Several colony hybridizations were performed, and two clones (SEQ ID NOS:1 and 3) were isolated. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOS:1 and 3.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. Similar to CD40, RANK associates with TRAF2 and TRAF3 (as determined by co-immunoprecipitation assays substantially as described by Rothe et al., *Cell* 83:1243, 1995). TRAFs are critically important in the regulation of the immune and inflammatory response. Through their association with various members of the TNF receptor superfamily, a signal is transduced to a cell. That signal results in the proliferation, differentiation or apoptosis of the cell, depending on which receptor(s) is/are triggered and which TRAF(s) associate with the receptor(s); different signals can be transduced to a cell via coordination of various signaling events. Thus, a signal transduced through one member of this family may be proliferative, differentiative or apoptotic, depending on other signals being transduced to the cell, and/or the state of differentiation of the cell. Such exquisite regulation of this proliferative/apoptotic pathway is necessary to develop and maintain protection against pathogens; imbalances can result in autoimmune disease.

RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic

signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response.

Moreover, the post-natal lethality of mice having a targeted disruption of the TRAF3 gene demonstrates the importance of this molecule not only in the immune response but in development. The isolation of RANK, as a protein that associates with TRAF3, and its ligand, RANKL, will allow further definition of this signaling pathway, and development of diagnostic and therapeutic modalities for use in the area of autoimmune and/or inflammatory disease.

10 **DNA_s, Proteins and Analogs**

The present invention provides isolated RANKL polypeptides and analogs (or muteins) thereof having an activity exhibited by the native molecule (i.e., RANKL muteins that bind specifically to a RANK expressed on cells or immobilized on a surface or to RANKL-specific antibodies; soluble forms thereof that inhibit RANK ligand-induced signaling through RANK). Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of RANKL within the scope of the invention also include various structural forms of the primary proteins which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a RANKL protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Derivatives of RANKL may also be obtained by the action of cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. The inventive proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, the proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the proteins or against other proteins which are similar to RANKL, as well as other proteins that bind RANKL or homologs thereof.

35 Soluble forms of RANKL are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the RANKL is shown in SEQ ID NOs:10 and 12 (murine and human, respectively). Computer analysis indicated that the RANKL is a Type 2 transmembrane protein; murine RANKL contains a predicted 48 amino acid

intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain, and human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO:9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID NO:10), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID NO:12).

Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID NO:12 (1 through 139 of SEQ ID NO:10). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID NO:12 (human RANKL; amino acids 48 and 139 of SEQ ID NO:10). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID NO:12 (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID NO:10). Those skilled in the art can prepare these and additional soluble forms through routine experimentation.

Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNF family (of which RANKL is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANKL proteins within the scope of this invention include covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide

sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

- 5 Protein fusions can comprise peptides added to facilitate purification or identification of RANKL proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *BioTechnology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

- Fusion proteins further comprise the amino acid sequence of a RANKL linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG, having a nucleotide 15 amino acid sequence set forth in SEQ ID NO:8. Fragments of an Fc region may also be used, as can Fc mutants. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to Fc γ RI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that Leu(234) and Leu(235) were critical to high affinity binding of IgG₃ to Fc γ RI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or 20 in combination, can be made in an IgG, Fc region to decrease the affinity of IgG, for Fc γ RI. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer 25 with as many as four RANKL regions.

- In another embodiment, RANKL proteins further comprise an oligomerizing peptide such as a leucine zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver 30 (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the 35 nuclear oncogenes *fos* and *jun* comprise leucine zipper domains preferentially form a

heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). The leucine zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, 5 coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The leucine zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the leucine zipper domains could contribute to the oligomeric 10 structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Leucine zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Leucine zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 15 254:539; 1991). The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a leucine zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (J. Mol. Biol. 98:293; 1975), in which residues *a* and *d* are generally hydrophobic 20 residues, with *d* being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical leucine zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

25 The leucine residues at position *d* contribute large hydrophobic stabilization energies, and are important for dimer formation (Krystek et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy et al. recently reported the synthesis of a triple-stranded α -helical bundle in which the helices run up-down (*Science* 259:1288, 1993). Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the 30 formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils.

Several studies have indicated that conservative amino acids may be substituted for individual leucine residues with minimal decrease in the ability to dimerize; multiple changes, however, usually result in loss of this ability (Landschulz et al., *Science* 35 243:1681, 1989; Turner and Tjian, *Science* 243:1689, 1989; Hu et al., *Science* 250:1400, 1990). van Heekeren et al. reported that a number of different amino residues can be substituted for the leucine residues in the leucine zipper domain of GCN4, and further found that some GCN4 proteins containing two leucine substitutions were weakly active.

- (*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 1992). None of the mutations affected the ability of MVF to form a tetramer.
- Amino acid substitutions in the *a* and *d* residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, CA). When all residues at position *a* are changed to isoleucine, the leucine zipper still forms a parallel dimer. When, in addition to this change, all leucine residues at position *d* are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all amino acids at position *d* with isoleucine and at position *a* with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains.
- The present invention also includes RANKL with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of RANKL protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn- $\text{A}_1\text{-Z}$, where A_1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A_1 and Z, or an amino acid other than Asn between Asn and A_1 .
- RANKL protein derivatives may also be obtained by mutations of the native RANKL or subunits thereof. A RANKL mutated protein, as referred to herein, is a polypeptide homologous to a native RANKL protein, but which has an amino acid sequence different from the native protein because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a mutated peptide may be easily determined by analyzing the ability of the mutated peptide to bind its counterstructure in a specific manner. Moreover, activity of RANKL analogs, muteins or

derivatives can be determined by any of the assays described herein (for example, induction of NF- κ B activation).

- Analog of the inventive proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.
- When a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Subunits of the inventive proteins may be constructed by deleting terminal or internal residues or sequences. Soluble forms of RANKL can be readily prepared and tested for their ability to induce NF- κ B activation. Polypeptides corresponding to the cytoplasmic regions, and fragments thereof (for example, a death domain) can be prepared by similar techniques. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of RANKL to proteins that have similar structures, as well as by performing structural analysis of the inventive RANKL proteins.
- Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the biological activity of RANKL (i.e., ability of the inventive proteins to bind antibodies to the corresponding native protein in substantially equivalent a manner, the ability to bind the counterstructure in substantially the same manner as the native protein, the ability to induce a RANKL signal, or ability to induce NF- κ B activation). Examples of conservative substitutions include substitution of amino acids outside of the binding domain(s) (either ligand/receptor or antibody binding areas for the extracellular domain, or regions that interact with other, intracellular proteins for the cytoplasmic domain), and substitution of amino acids that do not alter the secondary and/or tertiary structure of the native protein. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.
- Mutations in nucleotide sequences constructed for expression of analog proteins or fragments thereof must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the mRNA.

Not all mutations in the nucleotide sequence which encodes a RANKL protein or fragments thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to 5 provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants, random mutagenesis may be conducted and the expressed 10 mutated proteins screened for the desired activity. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

15 Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent NOS. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference 20 herein.

Additional embodiments of the inventive proteins include RANKL polypeptides encoded by DNAs capable of hybridizing to the DNAs of SEQ ID NO:10 or 12 under 25 moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANKL, or more preferably under stringent conditions (for example, hybridization in 6 X SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are degenerate to those which encode the RANKL. In one embodiment, 30 RANKL polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANKL protein as set forth in SEQ ID NOS:10 and 12. In a preferred embodiment, RANKL polypeptides are at least about 80% identical in amino acid sequence to the native form of RANKL; most preferred polypeptides are those that are at least about 90% identical to native RANKL.

35 Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For

fragments derived from the RANKL protein, the identity is calculated based on that portion of the RANKL protein that is present in the fragment.

The biological activity of RANKL analogs or muteins can be determined by testing the ability of the analogs or muteins to induce a signal through RANK, for example, activation of transcription as described in the Examples herein. Alternatively, suitable assays, for example, an enzyme immunoassay or a dot blot, employing an antibody that binds native RANKL, or a soluble form of RANK, can be used to assess the activity of RANKL analogs or muteins. Suitable assays also include, for example, assays that measure the ability of a RANKL peptide or mutein to bind cells expressing RANK, and/or the biological effects thereon. Such methods are well known in the art.

Fragments of the RANKL nucleotide sequences are also useful. In one embodiment, such fragments comprise at least about 17 consecutive nucleotides, preferably at least about 25 nucleotides, more preferably at least 30 consecutive nucleotides, of the RANKL DNA disclosed herein. DNA and RNA complements of such fragments are provided herein, along with both single-stranded and double-stranded forms of the RANKL DNAs of SEQ ID NOS:10 and 12, and those encoding the aforementioned polypeptides. A fragment of RANKL DNA generally comprises at least about 17 nucleotides, preferably from about 17 to about 30 nucleotides. Such nucleic acid fragments (for example, a probe corresponding to the extracellular domain of RANKL) are used as a probe or as primers in a polymerase chain reaction (PCR).

The probes also find use in detecting the presence of RANKL nucleic acids in *in vitro* assays and such procedures as Northern and Southern blots. Cell types expressing RANKL can be identified as well. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. For PCR, 5' and 3' primers corresponding to the termini of a desired RANKL DNA sequence are employed to amplify that sequence, using conventional techniques.

Other useful fragments of the RANKL nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target RANKL mRNA (sense) or RANKL DNA (antisense) sequences. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

Uses of DNAs, Proteins and Analogs

The RANKL DNAs, proteins and analogs described herein will have numerous uses, including the preparation of pharmaceutical compositions. For example, soluble forms of RANKL will be useful to transduce signal via RANK. RANKL compositions (both protein and DNAs) will also be useful in development of antibodies to RANKL, both

those that inhibit binding to RANK and those that do not. The inventive DNAs are useful for the expression of recombinant proteins, and as probes for analysis (either quantitative or qualitative) of the presence or distribution of RANKL transcripts.

- The inventive proteins will also be useful in preparing kits that are used to detect soluble RANK or RANKL, or monitor RANK-related activity, for example, in patient specimens. RANKL proteins will also find uses in monitoring RANK-related activity in other samples or compositions, as is necessary when screening for antagonists or mimetics of this activity (for example, peptides or small molecules that inhibit or mimic, respectively, the interaction). A variety of assay formats are useful in such kits, including (but not limited to) ELISA, dot blot, solid phase binding assays (such as those using a biosensor), rapid format assays and bioassays.

The purified RANKL according to the invention will facilitate the discovery of inhibitors of RANK, and thus, inhibitors of an inflammatory response (via inhibition of NF- κ B activation). The use of a purified RANKL polypeptide in the screening for potential inhibitors is important and can virtually eliminate the possibility of interfering reactions with contaminants. Such a screening assay can utilize either the extracellular domain of RANKL, or a fragment thereof. Detecting the inhibiting activity of a molecule would typically involve use of a soluble form of RANKL derived from the extracellular domain in a screening assay to detect molecules capable of binding RANK and inhibiting binding of the RANKL.

In addition, RANKL polypeptides can also be used for structure-based design of RANKL-inhibitors. Such structure-based design is also known as "rational drug design." The RANKL polypeptides can be three-dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance or homology modeling, all of which are well-known methods. The use of RANKL structural information in molecular modeling software systems to assist in inhibitor design is also encompassed by the invention. Such computer-assisted modeling and drug design may utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. A particular method of the invention comprises analyzing the three dimensional structure of RANKL for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

Moreover, as shown in the Examples herein, soluble forms of RANKL will be useful to induce maturation of dendritic cells (DC), and to enhance their allo-stimulatory capacity. Accordingly, RANKL proteins will be useful in augmenting an immune response, and can be used for these purposes either ex vivo (i.e., in obtaining cells such as DC from an individual, exposing them to antigen and cytokines ex vivo, and re-administering them to the individual) or in vivo (i.e., as a vaccine adjuvant that will augment humoral and/or cellular immunity). RANKL will also be useful promoting

viability of T cells in the presence of TGF β , which will also be helpful in regulating an immune response.

Expression of Recombinant RANKL.

5 The proteins of the present invention are preferably produced by recombinant DNA methods by inserting a DNA sequence encoding RANKL protein or an analog thereof into a recombinant expression vector and expressing the DNA sequence in a recombinant expression system under conditions promoting expression. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, provide a synthetic gene which is capable of being inserted in a recombinant expression vector and expressed in a recombinant transcriptional unit.

10 Recombinant expression vectors include synthetic or cDNA-derived DNA fragments encoding RANKL, or homologs, muteins or bioequivalent analogs thereof, operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, as described in detail below. The ability to 15 replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

20 DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the 25 polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA sequences encoding RANKL, or homologs or analogs thereof which are to be expressed in 30 a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

35 Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biote, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived

from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

- Promoters commonly used in recombinant microbial expression vectors include the 5 β -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (*trp*) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ *PL* 10 promoter and cl857ts thermostable repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ *PL* promoter include plasmid pHUB2, resident in *E. coli* strain IMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

- Suitable promoter sequences in yeast vectors include the promoters for 15 metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

- Preferred yeast vectors can be assembled using DNA sequences from pBR322 for 20 selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast α -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, 25 one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

- The transcriptional and translational control sequences in expression vectors to be 30 used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly

useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* II site located in the viral origin of replication is included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

- A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A preferred eukaryotic vector for expression of RANKL DNA is referred to as pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), and includes regulatory sequences derived from SV40, human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). Other preferred vectors include pDC409 and pDC410, which are derived from pDC406. pDC410 was derived from pDC406 by substituting the EBV origin of replication with sequences encoding the SV40 large T antigen. pDC409 differs from pDC406 in that a *Bgl* II restriction site outside of the multiple cloning site has been deleted, making the *Bgl* II site within the multiple cloning site unique.
- A useful cell line that allows for episomal replication of expression vectors, such as pDC406 and pDC409, which contain the EBV origin of replication, is CV-1/EBNA (ATCC CRL 10478). The CV-1/EBNA cell line was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter.

25 Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may express the desired protein (RANKL, its homologs or analogs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

Suitable host cells for expression of proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus* spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce proteins using RNAs

derived from the DNA constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

- 5 Prokaryotic expression hosts may be used for expression of RANKL, or homologs or analogs thereof that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.
- 10

- Recombinant RANKL may also be expressed in yeast hosts, preferably from the 15 *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of 20 replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then 25 provides an effective environment for detecting transformation by growth in the absence of tryptophan.

- Suitable yeast transformation protocols are known to those of skill in the art; an 30 exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for Trp⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μ g/ml adenine and 20 μ g/ml uracil. Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μ g/ml adenine and 80 μ g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

35 Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *BioTechnology* 6:47 (1988). Examples of

suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, CV-1/EBNA (ATCC CRL 10478), L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

10

Purification of Recombinant RANKL

Purified RANKL, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANKL and homologs thereof. For example, a RANKL expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANKL protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the RANKL protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANKL.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANKL composition.

Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

- 10 Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

- 15 Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive protein free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

20 **25 Uses and Administration of RANKL Compositions**

The present invention provides methods of using therapeutic compositions comprising an effective amount of a protein and a suitable diluent and carrier, and methods for regulating an immune or inflammatory response. The use of RANKL in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated.

30 For therapeutic use, purified protein is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, RANKL protein compositions administered to regulate immune function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANKL, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, 5 the desired response, the condition of the patient, and so forth.

As shown herein, RANKL has beneficial effects on various cells important in the immune system. Accordingly, RANKL may be administered to an individual as a vaccine adjuvant, or as a therapeutic agent to upregulate an immune response, for example, in infectious disease. Moreover, NF- κ B has been found to play a protective role in preventing apoptotic death of cells induced by TNF- α or chemotherapy. Accordingly, agonists of RANK (i.e., RANKL and agonistic antibodies) will be useful in protecting RANK-expressing cells from the negative effects of chemotherapy or the presence of high levels of TNF- α such as occur in sepsis (see, i.e., Barinaga, *Science* 274:724, 1996, and the articles by Beg and Baltimore and Wang et al., pages 782 and 784 of that same issue of *Science*). 10

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps". 25

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference. 30

EXAMPLE 1

30 The example describes the identification and isolation of a DNA encoding a novel member of the TNF receptor superfamily. A partial cDNA insert with a predicted open reading frame having some similarity to CD40 (a cell-surface antigen present on the surface of both normal and neoplastic human B cells that has been shown to play an important role in B-cell proliferation and differentiation; Stamenkovic et al., *EMBO J.* 8:1403, 1989), 35 was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendrite cells (DC). The insert was excised from the vector by restriction endonuclease digestion, gel purified, labeled with 32 P, and used to hybridize to colony blots generated from a DC cDNA library containing larger cDNA inserts using



high stringency hybridization and washing techniques (hybridization in 5xSSC, 50% formamide at 42°C overnight, washing in 0.5xSSC at 63°C); other suitable high stringency conditions are disclosed in Sambrook et al. in *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY: 1989), 9.52-9.55.

- 5 Initial experiments yielded a clone referred to as 9D-8A (SEQ ID NO:1); subsequent analysis indicated that this clone contained all but the extreme 5' end of a novel cDNA, with predicted intron sequence at the extreme 5' end (nucleotides 1-92 of SEQ ID NO:1). Additional colony hybridizations were performed, and a second clone was isolated. The second clone, referred to as 9D-15C (SEQ ID NO:3), contained the 5' end without intron 10 interruption but not the full 3' end. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

The encoded protein was designated RANK, for receptor activator of NF- κ B. The cDNA encodes a predicted Type I transmembrane protein having 616 amino acid residues, 15 with a predicted 24 amino acid signal sequence (the computer predicted cleavage site is after Leu24), a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail. The extracellular region of RANK displayed significant amino acid homology (38.5% identity, 52.3% similarity) to CD40. A cloning vector (pBluescriptSK-) containing human RANK sequence, designated 20 pBluescripthuRANK (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98285.

EXAMPLE 2

- 25 This example describes construction of a RANK DNA construct to express a RANK/Fc fusion protein. A soluble form of RANK fused to the Fc region of human IgG, was constructed in the mammalian expression vector pDC409 (USSN 08/571,579). This expression vector encodes the leader sequence of the Cytomegalovirus (CMV) open reading frame R27080 (SEQ ID NO:9), followed by amino acids 33-213 of RANK, followed by a 30 mutated form of the constant domain of human IgG, that exhibits reduced affinity for Fc receptors (SEQ ID NO:8; for the fusion protein, the Fc portion of the construct consisted of Arg3 through Lys232). An alternative expression vector encompassing amino acids 1-213 of RANK (using the native leader sequence) followed by the IgG, mutain was also prepared. Both expression vectors were found to induce high levels of expression of the 35 RANK/Fc fusion protein in transfected cells.

To obtain RANK/Fc protein, a RANK/Fc expression plasmid is transfected into CV-1/EBNA cells, and supernatants are collected for about one week. The RANK/Fc fusion protein is purified by means well-known in the art for purification of Fc fusion

proteins, for example, by protein A sepharose column chromatography according to manufacturer's recommendations (i.e., Pharmacia, Uppsala, Sweden). SDS-polyacrylamide gel electrophoresis analysis indicated that the purified RANK/Fc protein migrated with a molecular weight of ~55kDa in the presence of a reducing agent, and at a 5 molecular weight of ~110kDa in the absence of a reducing agent.

N-terminal amino acid sequencing of the purified protein made using the CMV R27080 leader showed 60% cleavage after Ala20, 20% cleavage after Pro22 and 20% cleavage after Arg28 (which is the Furin cleavage site; amino acid residues are relative to SEQ ID NO:9); N-terminal amino acid analysis of the fusion protein expressed with the native leader showed cleavage predominantly after Gln25 (80% after Gln25 and 20% after Arg23; amino acid residues are relative to SEQ ID NO:6, full-length RANK). Both fusion proteins were able to bind a ligand for RANK in a specific manner (i.e., they bound to the surface of various cell lines such as a murine thymoma cell line, EL4), indicating that the presence of additional amino acids at the N-terminus of RANK does not interfere with its 10 ability to bind RANKL. Moreover, the construct comprising the CMV leader encoded RANK beginning at amino acid 33; thus, a RANK peptide having an N-terminus at an amino acid between Arg23 and Pro33, inclusive, is expected to be able to bind a ligand for RANK in a specific manner.

Other members of the TNF receptor superfamily have a region of amino acids 20 between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between 196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids 25 for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:6, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 3

This example illustrates the preparation of monoclonal antibodies against RANK. 30 Preparations of purified recombinant RANK, for example, or transfected cells expressing high levels of RANK, are employed to generate monoclonal antibodies against RANK using conventional techniques, such as those disclosed in U.S. Patent 4,411,993. DNA encoding RANK can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in 35 interfering with RANK-induced signaling (antagonistic or blocking antibodies) or in inducing a signal by cross-linking RANK (agonistic antibodies), as components of

EXAMPLE 4

This example illustrates the induction of NF- κ B activity by RANK in 293/EBNA cells (cell line was derived by transfection of the 293 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) that constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter). Activation of NF- κ B activity was measured in 293/EBNA cells essentially as described by Yao et al. (*Immunity* 3:811, 1995). Nuclear extracts were prepared and analyzed for NF- κ B activity by a gel retardation assay using a 25 base pair oligonucleotide spanning the NF- κ B binding sites. Two million cells were seeded into 10 cm dishes two days prior to DNA transfection and cultured in 10 DMEM/F12 media containing 2.5% FBS (fetal bovine serum). DNA transfections were performed as described herein for the IL-8 promoter/reporter assays.

Nuclear extracts were prepared by solubilization of isolated nuclei with 400 mM NaCl (Yao et al., *supra*). Oligonucleotides containing an NF- κ B binding site were annealed and endlabeled with 32 P using T4 DNA polynucleotide kinase. Mobility shift reactions contained 10 μ g of nuclear extract, 4 μ g of poly(dI-dC) and 15,000 cpm labeled double-stranded oligonucleotide and incubated at room temperature for 20 minutes. Resulting protein-DNA complexes were resolved on a 6% native polyacrylamide gel in 0.25 X Tris-borate-EDTA buffer.

Overexpression of RANK resulted in induction of NF- κ B activity as shown by an appropriate shift in the mobility of the radioactive probe on the gel. Similar results were observed when RANK was triggered by a ligand that binds RANK and transduces a signal to cells expressing the receptor (i.e., by co-transfecting cells with human RANK and murine RANKL DNA; see Example 7 below), and would be expected to occur when triggering is done with agonistic antibodies.

EXAMPLE 5

This example describes a gene promoter/reporter system based on the human Interleukin-8 (IL-8) promoter used to analyze the activation of gene transcription *in vivo*. The induction of human IL-8 gene transcription by the cytokines Interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF- α) is known to be dependent upon intact NF- κ B and 30 NF-IL-6 transcription factor binding sites. Fusion of the cytokine-responsive IL-8 promoter with a cDNA encoding the murine IL-4 receptor (mIL-4R) allows measurement of promoter activation by detection of the heterologous reporter protein (mIL-4R) on the cell surface of transfected cells.

Human kidney epithelial cells (293/EBNA) are transfected (via the DEAE/DEXTRAN method) with plasmids encoding: 1). the reporter/promoter construct (referred to as pIL-8rep), and 2). the cDNA(s) of interest. DNA concentrations are always kept constant by the addition of empty vector DNA. The 293/EBNA cells are plated at a

density of 2.5×10^4 cells/ml (3 ml/well) in a 6 well plate and incubated for two days prior to transfection. Two days after transfection, the mIL-4 receptor is detected by a radioimmunoassay (RIA) described below.

- In one such experiment, the 293/EBNA cells were co-transfected with DNA 5 encoding RANK and with DNA encoding RANKL (see Example 7 below). Co-expression of this receptor and its counterstructure by cells results in activation of the signalling process of RANK. For such co-transfection studies, the DNA concentration/well for the DEAE transfection were as follows: 40 ng of pIL-8rep [pBluescriptSK- vector (Stratagene)]; 0.4 ng CD40 (DNA encoding CD40, a control receptor; pCDM8 vector); 0.4 ng RANK (DNA 10 encoding RANK; pDC409 vector), and either 1-50 ng CD40L (DNA encoding the ligand for CD40, which acts as a positive control when co-transfected with CD40 and as a negative control when co-transfected with RANK; in pDC304) or RANKL (DNA encoding a ligand for RANK; in pDC406). Similar experiments can be done using soluble RANKL or agonistic antibodies to RANK to trigger cells transfected with RANK.
- 15 For the mIL-4R-specific RIA, a monoclonal antibody reactive with mIL-4R is labeled with ^{125}I via a Chloramini T conjugation method; the resulting specific activity is typically 1.5×10^{16} cpm/mmol. After 48 hours, transfected cells are washed once with media (DMEM/F12 5% FBS). Non-specific binding sites are blocked by the addition of pre-warmed binding media containing 5% non-fat dry milk and incubation at 37°C/5% CO₂, 20 in a tissue culture incubator for one hour. The blocking media is decanted and binding buffer containing ^{125}I anti-mIL-4R (clone M1; rat IgG1) is added to the cells and incubated with rocking at room temperature for 1 hour. After incubation of the cells with the radio-labeled antibody, cells are washed extensively with binding buffer (2X) and twice with phosphate-buffered saline (PBS). Cells are lysed in 1 ml of 0.5M NaOH, and total 25 radioactivity is measured with a gamma counter.

- Using this assay, 293/EBNA co-transfected with DNAs encoding RANK demonstrated transcriptional activation, as shown by detection of mIL-4R on the cell surface. Overexpression of RANK resulted in transcription of mIL-4R, as did triggering of the RANK by RANKL. Similar results are observed when RANK is triggered by 30 agonistic antibodies.

EXAMPLE 6

- This example illustrates the association of RANK with TRAF proteins. Interaction of RANK with cytoplasmic TRAF proteins was demonstrated by co-immunoprecipitation assays essentially as described by Hsu et al. (*Cell* 84:299; 1996). Briefly, 293/EBNA cells 35 were co-transfected with plasmids that direct the synthesis of RANK and epitope-tagged (FLAG6; SEQ ID NO:7) TRAF2 or TRAF3. Two days after transfection, surface proteins

were labeled with biotin-ester, and cells were lysed in a buffer containing 0.5% NP-40. RANK and protein associated with this receptor were immunoprecipitated with anti-RANK, washed extensively, resolved by electrophoretic separation on a 6-10% SDS polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane for 5 Western blotting. The association of TRAF2 and TRAF3 proteins with RANK was visualized by probing the membrane with an antibody that specifically recognizes the FLAG® epitope. TRAFs 2 and 3 did not immunoprecipitate with anti-RANK in the absence of RANK expression.

EXAMPLE 7

10 This example describes isolation of a ligand for RANK, referred to as RANKL, by direct expression cloning. The ligand was cloned essentially as described in USSN 08/249,189, filed May 24, 1994 (the relevant disclosure of which is incorporated by reference herein), for CD40L. Briefly, a library was prepared from a clone of a mouse thymoma cell line EL-4 (ATCC TIB 39), called EL-40.5, derived by sorting five times with biotinylated CD40/Fc fusion protein in a FACS (fluorescence activated cell sorter). The cDNA library was made using standard methodology; the plasmid DNA was isolated and transfected into sub-confluent CV1-EBNA cells using a DEAE-dextran method. Transfectants were screened by slide autoradiography for expression of RANKL using a two-step binding method with RANK/Fc fusion protein as prepared in Example 2 followed by radioiodinated goat anti-human IgG antibody.

15 A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 25 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in US patent 5,599,905, issued February 4, 1997); the 5' region was found to be identical to that of 30 human RANKL as shown in SEQ ID NO: 12, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9.

This ligand is useful for assessing the ability of RANK to bind RANKL by a number of different assays. For example, transfected cells expressing RANKL can be used in a FACS assay (or similar assay) to evaluate the ability of soluble RANK to bind 35 RANKL. Moreover, soluble forms of RANKL can be prepared and used in assays that are known in the art (i.e., ELISA or BiAcore assays essentially as described in USSN 08/249,189, filed May 24, 1994). RANKL is also useful in affinity purification of RANK,

and as a reagent in methods to measure the levels of RANK in a sample. Soluble RANKL is also useful in inducing NF- κ B activation and thus protecting cells that express RANK from apoptosis.

EXAMPLE 8

5 This example describes the isolation of a human RANK ligand (RANKL) using a PCR-based technique. Murine RANK ligand-specific oligonucleotide primers were used in PCR reactions using human cell line-derived first strand cDNAs as templates. Primers corresponded to nucleotides 478-497 and to the complement of nucleotides 858-878 of murine RANK ligand (SEQ ID NO:10). An amplified band approximately 400 bp in length
10 from one reaction using the human epidermoid cell line KB (ATCC CCL-17) was gel purified, and its nucleotide sequence determined; the sequence was 85% identical to the corresponding region of murine RANK ligand, confirming that the fragment was from human RANKL.

To obtain full-length human RANKL cDNAs, two human RANKL-specific
15 oligonucleotides derived from the KB PCR product nucleotide sequence were radiolabeled and used as hybridization probes to screen a human PBL cDNA library prepared in lambda gt10 (Stratagene, La Jolla, CA), substantially as described in US patent 5,599,905, issued February 4, 1997. Several positive hybridizing plaques were identified and purified, their inserts subcloned into pBluescript SK⁺ (Stratagene, La Jolla, CA), and their nucleotide
20 sequence determined. One isolate, PBL3, was found to encode most of the predicted human RANKL, but appeared to be missing approximately 200 bp of 5' coding region. A second isolate, PBL5 was found to encode much of the predicted human RANKL, including the entire 5' end and an additional 200 bp of 5' untranslated sequence.

The 5' end of PBL5 and the 3' end of PBL3 were ligated together to form a full
25 length cDNA encoding human RANKL. The nucleotide and predicted amino acid sequence of the full-length human RANK ligand is shown in SEQ ID NO:12. Human RANK ligand shares 83% nucleotide and 84% amino acid identity with murine RANK ligand. A plasmid vector containing human RANKL sequence, designated pBluescript-huRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD
30 (ATCC) on March 11, 1997 under terms of the Budapest Treaty, and given accession number 98354.

Murine and human RANKL are Type 2 transmembrane proteins. Murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain. Human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

EXAMPLE 9

This example describes the chromosomal mapping of human RANK using PCR-based mapping strategies. Initial human chromosomal assignments were made using RANK and RANKL-specific PCR primers and a BIOS Somatic Cell Hybrid PCRable DNA kit from BIOS Laboratories (New Haven, CT), following the manufacturer's instructions. RANK mapped to human chromosome 18; RANK ligand mapped to human chromosome 13. More detailed mapping was performed using a radiation hybrid mapping panel Genebridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, AL; described in Walter, MA et al., *Nature Genetics* 7:22-28, 1994). Data from this analysis was then submitted electronically to the MIT Radiation Hybrid Mapper (URL: <http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) following the instructions contained therein. This analysis yielded specific genetic marker names which, when submitted electronically to the NCBI Entrez browser (URL: <http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=c&form=0>), yielded the specific map locations. RANK mapped to chromosome 18q22.1, and RANKL mapped to chromosome 13q14.

EXAMPLE 10

This example illustrates the preparation of monoclonal antibodies against RANKL. Preparations of purified recombinant RANKL, for example, or transfected cells expressing high levels of RANKL, are employed to generate monoclonal antibodies against RANKL using conventional techniques, such as those disclosed in US Patent 4,411,993. DNA encoding RANKL can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANKL signaling (antagonistic or blocking antibodies), as components of diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL.

To immunize rodents, RANKL immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intramuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent

- assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.
- Hybridoma clones thus generated can be screened by ELISA for reactivity with
- 10 RANKL, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in US Patent 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody.
- 15 The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANKL protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANKL and inhibit binding to RANK) and non-blocking (i.e., antibodies that bind RANKL and do not inhibit binding) are isolated.

EXAMPLE 11

This example demonstrates that RANK expression can be up-regulated. Human peripheral blood T cells were purified by flow cytometry sorting or by negative selection using antibody coated beads, and activated with anti-CD3 (OKT3, Dako) coated plates or phytohemagglutinin in the presence or absence of various cytokines, including Interleukin-4 (IL-4), Transforming Growth Factor- β (TGF- β) and other commercially available cytokines (IL-1 α , IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN- γ , TNF- α). Expression of RANK was evaluated by FACS in a time course experiment for day 2 to day 8, using a mouse monoclonal antibody mAb144 (prepared as described in Example 3), as shown in the table below. Results are expressed as '+' to '++++' referring to the relative increase in intensity of staining with anti-RANK. Double labeling experiments using both anti-RANK and anti-CD8 or anti-CD4 antibodies were also performed.

Table I: Upregulation of RANK by Cytokines

Cytokine (concentration)	Results:
IL-4 (50 ng/ml)	+
TGF- β (5 ng/ml)	+ to ++
IL-4 (50 ng/ml) + TGF- β (5 ng/ml)	++++
IL-1 α (10ng/ml)	-
IL-2 (20ng/ml)	-
IL-3 (25ng/ml)	-
IL-7 (20ng/ml)	-
IL-8 (10ng/ml)	-
IL-10 (30ng/ml)	-
IL-12 (10ng/ml)	-
IL-15 (10ng/ml)	-
IFN- γ (100U/ml)	-
TNF- α (10ng/ml)	-

- 15 Of the cytokines tested, IL-4 and TGF- β increased the level of RANK expression on both CD8+ cytotoxic and CD4+ helper T cells from day 4 to day 8. The combination of IL-4 and TGF- β acted synergistically to upregulate expression of this receptor on activated T cells. This particular combination of cytokines is secreted by suppressor T cells, and is believed to be important in the generation of tolerance (reviewed in Mitchison and Sieper,
- 20 Z. *Rheumatol.* 54:141, 1995), implicating the interaction of RANK in regulation of an immune response towards either tolerance or induction of an active immune response.

EXAMPLE 12

This example illustrates the influence of RANK.Fc and hRANKL on activated T cell growth. The addition of TGF β to anti-CD3 activated human peripheral blood T lymphocyte induces proliferation arrest and ultimately death of most lymphocytes within the first few days of culture. We tested the effect of RANK:RANKL interactions on TGF β -treated T cells by adding RANK.Fc or soluble human RANKL to T cell cultures.

Human peripheral blood T cells (7×10^3 PBT) were cultured for six days on anti-CD3 (OKT3, 5 μ g/ml) and anti-Flag (M1, 5 μ g/ml) coated 24 well plates in the presence of TGF β (1ng/ml) and IL-4 (10ng/ml), with or without recombinant FLAG-tagged soluble hRANKL (1 μ g/ml) or RANK.Fc (10 μ g/ml). Viable T cell recovery was determined by triplicate trypan blue countings.

The addition of RANK.Fc significantly reduced the number of viable T cells recovered after six days, whereas soluble RANKL greatly increased the recovery of viable T cells (Figure 1). Thus, endogenous or exogenous RANKL enhances the number of viable T cells generated in the presence of TGF β . TGF β , along with IL-4, has been implicated in immune response regulation when secreted by the T_H3/regulatory T cell subset. These T cells are believed to mediate bystander suppression of effector T cells. Accordingly, RANK and its ligand may act in an auto/paracrine fashion to influence T cell tolerance. Moreover, TGF β is known to play a role in the evasion of the immune system effected by certain pathogenic or opportunistic organisms. In addition to playing a role in the development of tolerance, RANK may also play a role in immune system evasion by pathogens.

25

EXAMPLE 13

This example illustrates the influence of the interaction of RANK on CD1a $^+$ dendritic cells (DC). Functionally mature dendritic cells (DC) were generated *in vitro* from CD34 $^+$ bone marrow (BM) progenitors. Briefly, human BM cells from normal healthy volunteers were density fractionated using Ficoll medium and CD34 $^+$ cell immunoaffinity isolated using an anti-CD34 matrix column (Ceprate, CellPro). The CD34 $^+$ BM cells were then cultured in human GM-CSF (20 ng/ml), human IL-4 (20 ng/ml), human TNF- α (20 ng/ml), human CHO-derived Flt3L (FL; 100 ng/ml) in Super McCoy's medium supplemented with 10% fetal calf serum in a fully humidified 37°C incubator (5% CO $_2$) for 14 days. CD1a $^+$, HLA-DR $^+$ DC were then sorted using a FACStar Plus™, and used for biological evaluation of RANK.

On human CD1a $^+$ DC derived from CD34 $^+$ bone marrow cells, only a subset (20-30%) of CD1a $^+$ DC expressed RANK at the cell surface as assessed by flow cytometric

analysis. However, addition of CD40L to the DC cultures resulted in RANK surface expression on the majority of CD1a⁺ DC. CD40L has been shown to activate DC by enhancing *in vitro* cluster formation, inducing DC morphological changes and upregulating HLA-DR, CD54, CD58, CD80 and CD86 expression

- 5 Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effect seen with CD40L (Figure 2). Sorted human CD1a⁺ DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- α and FL) (upper left panel), in cocktail plus CD40L (1 μ g/ml) (upper right), in cocktail plus RANKL (1 μ g/ml) (lower left), or in cocktail plus heat inactivated (aH) RANKL (1 μ g/ml) (lower right) in 24-well flat bottomed culture plates in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a,
10 CD54 or CD58.

20 The addition of RANKL to CD1a⁺ DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (Figure 3). Allogeneic T cells (1 \times 10⁵) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above for Figure 2 in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [³H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase β counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean \pm SD of triplicate cultures. Heat
25 inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the *in vitro* growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the
30 expression of CD80 or CD86.

35 RANKL can enhance DC cluster formation and functional capacity without modulating known molecules involved in cell adhesion (CD18, CD54), antigen presentation (HLA-DR) or costimulation (CD86), all of which are regulated by CD40/CD40L signaling. The lack of an effect on the expression of these molecules suggests that RANKL may regulate DC function via an alternate pathway(s) distinct from CD40/CD40L. Given that CD40L regulates RANK surface expression on *in vitro*-generated DC and that CD40L is upregulated on activated T cells during DC-T cell

interactions, RANK and its ligand may form an important part of the activation cascade that is induced during DC-mediated T cell expansion. Furthermore, culture of DC in RANKL results in decreased levels of CD11b expression, and increased levels of CD83. Both of these molecules are similarly modulated during DC maturation by CD40L (Cauz et al. J. 5 *Exp. Med.* 180:1263; 1994), indicating that RANKL induces DC maturation.

Dendritic cells are referred to as "professional" antigen presenting cells, and have a high capacity for sensitizing MHC-restricted T cells. There is growing interest in using dendritic cells *ex vivo* as tumor or infectious disease vaccine adjuvants (see, for example, Romani, et al., *J. Exp. Med.*, 180:83, 1994). Therefore, an agent such as RANKL, that 10 induces DC maturation and enhances the ability of dendritic cells to stimulate an immune response is likely to be useful in immunotherapy of various diseases.

EXAMPLE 14

This example describes the isolation of the murine homolog of RANK, referred to 15 as muRANK. MuRANK was isolated by a combination of cross-species PCR and colony hybridization. The conservation of Cys residues in the Cys-rich pseudorepeats of the extracellular domains of TNFR superfamily member proteins was exploited to design human RANK-based PCR primers to be used on murine first strand cDNAs from various sources. Both the sense upstream primer and the antisense downstream primer were 20 designed to have their 3' ends terminate within Cys residues.

The upstream sense primer encoded nucleotides 272-295 of SEQ ID NO:5 (region encoding amino acids 79-86); the downstream antisense primer encoded the complement of nucleotides 409-427 (region encoding amino acids 124-130). Standard PCR reactions were set up and run, using these primers and first strand cDNAs from various murine cell 25 line or tissue sources. Thirty reaction cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 20 seconds were run. PCR products were analyzed by electrophoresis, and specific bands were seen in several samples. The band from one sample was gel purified and DNA sequencing revealed that the sequence between the primers was approximately 85% identical to the corresponding human RANK nucleotide sequence.

A plasmid based cDNA library prepared from the murine fetal liver epithelium line FLE18 (one of the cell lines identified as positive in the PCR screen) was screened for full-length RANK cDNAs using murine RANK-specific oligonucleotide probes derived from the murine RANK sequence determined from sequencing the PCR product. Two cDNAs, one encoding the 5' end and one encoding the 3' end of full-length murine RANK (based 30 on sequence comparison with the full-length human RANK) were recombined to generate a full-length murine RANK cDNA. The nucleotide and amino acid sequence of muRANK are shown in SEQ ID Nos:14 and 15.

The cDNA encodes a predicted Type 1 transmembrane protein having 625 amino acid residues, with a predicted 30 amino acid signal sequence, a 184 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 390 amino acid cytoplasmic tail. The extracellular region of muRANK displayed significant amino acid homology (69.7% identity, 80.8% similarity) to huRANK. Those of skill in the art will recognize that the actual cleavage site can be different from that predicted by computer; accordingly, the N-terminal of RANK may be from amino acid 25 to amino acid 35.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 214, and 197 of SEQ ID NO:14, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 15

This example illustrates the preparation of several different soluble forms of RANK and RANKL. Standard techniques of restriction enzyme cutting and ligation, in combination with PCR-based isolation of fragments for which no convenient restriction sites existed, were used. When PCR was utilized, PCR products were sequenced to ascertain whether any mutations had been introduced; no such mutations were found.

In addition to the huRANK/Fc described in Example 2, another RANK/Fc fusion protein was prepared by ligating DNA encoding amino acids 1-213 of SEQ ID NO:6, to DNA encoding amino acids 3-232 of the Fc mutein described previously (SEQ ID NO:8). A similar construct was prepared for murine RANK, ligating DNA encoding amino acids 1-213 of full-length murine RANK (SEQ ID NO:15) to DNA encoding amino acids 3-232 of the Fc mutein (SEQ ID NO:8).

A soluble, tagged, poly-His version of huRANKL was prepared by ligating DNA encoding the leader peptide from the immunoglobulin kappa chain (SEQ ID NO:16) to DNA encoding a short version of the FLAG™ tag (SEQ ID NO:17), followed by codons encoding Gly Ser, then a poly-His tag (SEQ ID NO:18), followed by codons encoding Gly Thr Ser, and DNA encoding amino acids 138-317 of SEQ ID NO:13. A soluble, poly-His tagged version of murine RANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to codons encoding Arg Thr Ser, followed by DNA encoding poly-His (SEQ ID NO:18) followed by DNA encoding amino acids 119-294 of SEQ ID NO:11.

A soluble, oligomeric form of huRANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to a codon encoding Asp followed by DNA ending a trimer-former "leucine" zipper (SEQ ID NO:19), then by codons encoding Thr Arg Ser followed by amino acids 138-317 of SEQ ID NO:13.

- 5 These and other constructs are prepared by routine experimentation. The various DNAs are then inserted into a suitable expression vector, and expressed. Particularly preferred expression vectors are those which can be used in mammalian cells. For example, pDC409 and pDC304, described herein, are useful for transient expression. For stable transfection, the use of CHO cells is preferred; several useful vectors are described in
10 USSN 08/785,150, now allowed, for example, one of the 2A5-3 λ-derived expression vectors discussed therein.

EXAMPLE 16

- This example demonstrates that RANKL expression can be up-regulated on murine T cells. Cells were obtained from mesenteric lymph nodes of C57BL/6 mice, and activated with anti-CD3 coated plates, Concanavalin A (ConA) or phorbol myristate acetate in combination with ionomycin (anti-CD3: 500A2; Immunex Corporation, Seattle WA; ConA, PMA, ionomycin, Sigma, St. Louis, MO) substantially as described herein, and cultured from about 2 to 5 days. Expression of RANKL was evaluated in a three color analysis by
15 FACS, using antibodies to the T cell markers CD4, CD8 and CD45RB, and RANK/Fc, prepared as described herein.

RANKL was not expressed on unstimulated murine T cells. T cells stimulated with either anti-CD3, ConA, or PMA/ionomycin, showed differential expression of RANKL: CD4⁺/CD45RB^{Lo} and CD4⁺/CD45RB^{Hi} cells were positive for RANKL, but CD8+ cells
20 were not. RANKL was not observed on B cells, similar to results observed with human cells.

EXAMPLE 17

- This example illustrates the effects of murine RANKL on cell proliferation and activation. Various cells or cell lines representative of cells that play a role in an immune response (murine spleen, thymus and lymphnode) were evaluated by culturing them under conditions promoting their viability, in the presence or absence of RANKL. RANKL did not stimulate any of the tested cells to proliferate. One cell line, a macrophage cell line referred to as RAW 264.7 (ATCC accession number TIB 71) exhibited some signs of
30 activation.
35

RAW cells constitutively produce small amounts of TNF-α. Incubation with either human or murine RANKL enhanced production of TNF-α by these cells in a dose

dependent manner. The results were not due to contamination of RANKL preparations with endotoxin, since boiling RANKL for 10 minutes abrogated TNF- α production, whereas a similar treatment of purified endotoxin (LPS) did not affect the ability of the LPS to stimulate TNF- α production. Despite the fact that RANKL activated the macrophage cell line RAW T64.7 for TNF- α production, neither human RANKL nor murine RANKL stimulated nitric oxide production by these cells.

EXAMPLE 18

This example illustrates the effects of murine RANKL on growth and development 10 of the thymus in fetal mice. Pregnant mice were injected with 1 mg of RANK/Fc or vehicle control protein (murine serum albumin; MSA) on days 13, 16 and 19 of gestation. After birth, the neonates continued to be injected with RANK/Fc intraperitoneally (IP) on a daily basis, beginning at a dose of 1 μ g, and doubling the dose about every four days, for a final dosage of 4 μ g. Neonates were taken at days 1, 8 and 15 post birth, their thymuses and 15 spleens harvested and examined for size, cellularity and phenotypic composition.

A slight reduction in thymic size at day 1 was observed in the neonates born to the female injected with RANK/Fc; a similar decrease in size was not observed in the control neonates. At day 8, thymic size and cellularity were reduced by about 50% in the RANK/Fc-treated animals as compared to MSA treated mice. Phenotypic analysis 20 demonstrated that the relative proportions of different T cell populations in the thymus were the same in the RANK/Fc mice as the control mice, indicating that the decreased cellularity was due to a global depression in the number of thymic T cells as opposed to a decrease in a specific population(s). The RANK/Fc-treated neonates were not significantly different from the control neonates at day 15 with respect to either size, cellularity or phenotype of 25 thymic cells. No significant differences were observed in spleen size, cellularity or composition at any of the time points evaluated. The difference in cellularity on day 8 and not on day 15 may suggest that RANK/Fc may assert its effect early in thymic development.

30

EXAMPLE 19

This example demonstrates that the C-terminal region of the cytoplasmic domain of RANK is important for binding of several different TRAF proteins. RANK contains at 35 least two recognizable PXQX(Q)T motifs that are likely TRAF docking sites. Accordingly, the importance of various regions of the cytoplasmic domain of RANK for TRAF binding was evaluated. A RANK/GST fusion protein was prepared substantially as described in Smith and Johnson, *Gene* 67:31 (1988), and used in the preparation of various truncations as described below.

Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed 5 to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 1 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs.

Radio-labeled (³⁵S-Met, Cys) TRAF proteins were prepared by *in vitro* translation using a commercially available reticulocyte lysate kit according to manufacturer's 10 instructions (Promega). Truncated GST fusion proteins were purified substantially as described in Smith and Johnson (supra). Briefly, *E. coli* were transfected with an expression vector encoding a fusion protein and induced to express the protein. The bacteria were lysed, insoluble material removed, and the fusion protein isolated by precipitation with glutathione-coated beads (Sepharose 4B, Pharmacia, Uppsala Sweden).
 15 The beads were washed, and incubated with various radiolabeled TRAF proteins. After incubation and wash steps, the fusion protein/TRAF complexes were removed from the beads by boiling in 0.1% SDS + β -mercaptoethanol, and loaded onto 12% SDS gels (Novex). The gels were subjected to autoradiography, and the presence or absence of radiolabeled material recorded. The results are shown in Table 2 below.
 20

Table 2: Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK

C terminal Truncations:	E206-S339	E206-Y421	E206-M476	E206-G544	Full length
TRAF1	-	-	-	-	++
TRAF2	-	-	-	-	++
TRAF3	-	-	-	-	++
TRAF4	-	-	-	-	-
TRAF5	-	-	-	-	+
TRAF6	-	+	+	+	++

25

These results indicate that TRAF1, TRAF2, TRAF3, TRAF 5 and TRAF6 bind to the most distal portion of the RANK cytoplasmic domain (between amino-acid G544 and A616). TRAF6 also has a binding site between S339 and Y421. In this experiment, TRAF5 also bound the cytoplasmic domain of RANK.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Immunex Corporation

(ii) TITLE OF INVENTION: Ligand for Receptor Activator of NF-kappaB

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Immunex Corporation, Law Department
(B) STREET: 51 University Street
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98101

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Power Macintosh
(C) OPERATING SYSTEM: Apple Operating System 7.5.5
(D) SOFTWARE: Microsoft Word for Power Macintosh 6.0.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 22 DECEMBER 1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: USSN 60/064,671
(B) FILING DATE: 14 OCTOBER 1997
(C) CLASSIFICATION:

(viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: USSN 08/813,509
(B) FILING DATE: 07 MARCH 1997
(C) CLASSIFICATION:

(ix) ATTORNEY/AGENT INFORMATION:

(A) NAME: Perkins, Patricia Anne
(B) REGISTRATION NUMBER: 34,693
(C) REFERENCE/DOCKET NUMBER: 2052-WO

(x) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (206)587-0430
(B) TELEFAX: (206)233-0644

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3115 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS

(B) CLONE: 9D-8A

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 93..1868

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTGCTGCTG CTCTGGGGCG	TGCTCGCCCG GCTGCGAGTTT	TATCCAGAAA GAGCTTGTTG	60
GACTCTCTGC CTGACCTCTAG	TGTTCTTTTC AG GNG CCT TTG CAG ATC GCT CCT	Val Ala Leu Gln Ile Ala Pro	113
	1 15 20	3	
CCA TGT ACC AGT GAG AAC CAT	TAT GAG CAT CTG GGA CGG TGC TGT AAC		161
Pro Cys Thr Ser Gln Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn	10 15 20		
AAA TGT GAA CCA CGA AAQ TAC ATG TCT TGT AAA TGC ACT ACT ACC TCT			209
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Ser	25 30 35		
GAC AGT GTA TGT CTG CCC TGT GGC CGG GAT GAA TAC TTG GAT ACC TGG			257
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp	40 45 50		
AAT GAA GAA GAT AAA TCC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG			305
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys	60 65 70		
GCC CTG GTG GCC GTG GTC GGC GGC AAC ACC ACC CCC TGG CGC TGC			353
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys	75 80 85		
GCG TGC ACG CCT GGG TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC GGC			401
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg	90 95 100		
CCC AAC ACC GAG TCC CGG CGG GGC CTG GGC GGC CAG CAC CCC TTG CAG			449
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gin His Pro Leu Gln	105 110 115		
CTC AAC AAG GAC ACA GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT			497
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser	120 125 130 135		

GAT GCC TTT TCC TCC ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC
Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
140 145 150 545

TTC CTT CGA AAC AGA GCA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG
Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
155 160 165 593

GTT TGC AGT TCT CTG CCA GCT AGA AAA CCA CCA AAA GAA CCC CAT
Val Cys Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
170 175 180 641

GTT TGC TTG CCC GGT TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC
Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
185 190 195 689

CTG GTC GCT GCC ATC ATC TTT GGC GGT TGC TAT AGG AAA AAA CGG AAA
Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
200 205 210 215 737

GCA CTC ACA GCTT ATT TTG TGG CAC TGG ATC ATT AAC GAG GCT TGT GGC CGC
Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
220 225 230 785

CTA AGT GGA GAT AAC GAG TCC TCA GGT GAC AGT TGT GTC ACT ACA CAC
Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
235 240 245 833

ACG GCA AAC TTT GGT CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG
Thr Ala Asn Phe Gly Glu Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
250 255 260 881

ACT CTG GAG GAG AAC TTT CCA GAA GAT ATG TGC TAC CCA GAT CNA
Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
265 270 275 929

GGT GGT GTC TGT CAG GGC AGC TGT GTC GGA GGT GGT CCC TAC GCA CAA
Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Pro Tyr Ala Gln
280 285 290 295 977

GGC GAA GAT GCC AGG ATG CTC TCA TTG CTC ACT AAC ACC GAG ATA GAG
Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
300 305 310 1025

GAA GAC AGC TTC AGA CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG
Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
315 320 325 1073

CCC TCC CAG CCC ACA GAC CAG TTA CTG TCC CTC ACT GAG CCT GGA AGC
Pro Ser Gln Pro Thr Asp Gln Leu Phe Leu Thr Ser Lys Pro Gly Ser
330 335 340 1121

AAA TCC ACA CCT OCT TTC TCT GAA CCC CTG GAG GTC GGG GAG AAT GAC
Lys Ser Thr Pro Pro Phe Ser Gln Pro Leu Glu Val Gly Glu Asn Asp
345 350 355 1169

AGT TTA AGC CAG TGC TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA
Ser Leu Ser Gln Cys Phe Thr Gly Thr Glu Ser Thr Val Glu Ser Glu
360 365 370 375 1217

ACC TCC AAC TCC ACT GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met 1265
 380 385 390
 TCC TCT GAA AAC TAC TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro 1313
 395 400 405
 CAC TGG GCA GCC AGC CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC
 His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly 1361
 410 415 420
 TGG CGG AAC CCT CCT GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro 1409
 425 430 435
 AAA CGT GGA CCC TTG CCC CAG TGC GGC TAT GGC ATG GGC CCTT CCC CCT
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro 1457
 440 445 450 455
 GAA GAA GAA GCC AGC AGG ACG GAG GGC AGC GAC CAG CCC GAG GAT GGG
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly 1505
 460 465 470
 GCT GAT GGG AGG CTC CCA AGC TCA CGG AGG GCA GGT GGC CGG TCT GGA
 Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly 1553
 475 480 485
 AGC TCC CCT GGT GGC CAG TCC CCT GCA TCT GGA ATT GTG ACT GGA AAC
 Ser Ser Pro Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn 1601
 490 495 500
 AGT AAC TCC ACG TTC ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC
 Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly 1649
 505 510 515
 GAC ATC ATC GTG GTC TAC GTC AGC CAG ACC TCG CAG GAG GGC GGC
 Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala 1697
 520 525 530 535
 GCG GCT GCG GAG CCC ATG GGC CGC CGG GTG CAG CAG GAG ACC CTG GCG
 Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala 1745
 540 545 550
 CGC CGA GAC TCC TTC GCG GGG AAC GGC CGC CGC TTC CGG GAC CGC TGC
 Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys 1793
 555 560 565
 GGC GGC CCC GAG GGG CTG CGG GAG CGC AGG AAG GGC TCG AGG CGG GTG
 Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val 1841
 570 575 580
 CAG GAG CAA GGC GGG GCC AAG GCT TGA GCGCCCCCCCAG TGGCTGGAG 1888
 Gln Glu Gln Gly Gly Ala Lys Ala 585
 590
 CCCGAAAGCTC GGAGGCCAGGG CTCGCGAGGG CACGCCCGCA GCGCTCTGCCG CAGCCCCCGC 1948
 CACCCGGAGG TCCGATCGGTA CAGTCGAGGA AGAACCAACCG GCAATTCTCTG CCCACTTTC 2008
 CTTCCAGAAA ATGGGCTTTT CAGGAAGTGA ATTGATGAGG ACTGUTCCCCA TGCCCCACGGA 2068

TGCTCAGAG CGCGGGGAC TGGGGAGAT GTTCCCCCTG CCACTCCCTCA AACCTGGAGC 2128
 AGTAATTGT GGCACTATGA CAGCTATT TTATGACTATC CTGTTCTGTO GGGGGGGGT 2188
 CTATGTTTC CCCCCATATT TTGTAFTCCCTT TTCAATRACTT TTCTTGATAT CTPTGCCTCCC 2248
 TCTTTTTAA TGTAAAGGTT TTCTCAAAAA TTCTCCAAA GTGAGGGTC TCTTTCCTTT 2308
 CTCTTTTCCC TTCTTTTTC TTCTTTGGC AACCTGGCNC TGGCCCAAGGC TAGAGTCAG 2368
 TGGTGGGATT ATAGCCCCGGT GCAGCCCTCA ACTCTGGGC TCAAGCAATC CAAGTGATCC 2428
 TCCACCTCA ACCTTGGAG TAGCTGGAT CACAGCTGCA GCGCACGCC AGCTTCCCTC 2488
 CCCCGACTCC CCCCCCCCAG AGACACGOTC CCACCATGTT ACCCAGGCTG GTCTCAAAC 2548
 CCCCACTAA AGCAGTCCTC CAGCTCGGC CTCCCAAAAGT ACTGGGATTA CAGGGCTGAG 2608
 CCCCCAGCTC GCGCTGGTTT ACGTTTTTC TTCTTGCGC CTGCTCACAG TTCTTAGAG 2668
 ATGGCTTC CAGTGGTGT CTATGTTAAGA CACTTTGGG AAAGGGCTAA ACATOTGAGG 2728
 CCTGGAGATA GTGCTAACT TGCTAGAAC ATGTTGGTGT ACTTTCATAT TTGAAAAT 2788
 GTTCTATATT CTCTTTC TAAAGGAG AAAAGGAGA ACCGATTTA TTTCCTCTGA 2848
 ATCTTTTAA GTTGTGGG TTCTTAAAGC AGACTAAGC TGAGTATGTC ACCTTACCCG 2908
 CTAGGGGGT AATTTATCCA TCTGGCAGA GCGACTCAGG TACTTGGTAA GCAATTTCT 2968
 AAAACTCCAA GTGCTSCAG CTGGGATTC TCTTATTTCT AGAGGTTCT CTGGAAAAGA 3028
 TGGAGAAAAT GAACAGGACA TGGGGCTCC? GGAAAGAAAG GCGCCGGAA GTTCAAGGA 3088
 GAATAAGTT GAAATTTAA AAAAAA 3115

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 591 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val	Ala	Leu	Gln	Ile	Ala	Pro	Pro	Cys	Thr	Ser	Glu	Lys	His	Tyr	Glu
1				5					10				15		
His	Leu	Gly	Arg	Cys	Cys	Asn	Lys	Cys	Glu	Pro	Gly	Lys	Tyr	Met	Ser
				20			25					30			
Ser	Lys	Cys	Thr	Thr	Thr	Ser	Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro
					35		40		45						
Asp	Glu	Tyr	Leu	Asp	Ser	Trp	Asn	Glu	Asp	Lys	Cys	Leu	Leu	His	
				50		55		60							

Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val Val Ala Gly Asn
 65 70 75 80
 Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly Tyr His Trp Ser
 85 90 95
 Gln Asp Cys Glu Cys Cys Cys Arg Arg Asn Thr Glu Cys Ala Pro Gly Leu
 100 105 110
 Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr Val Cys Lys Pro
 115 120 125
 Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser Thr Asp Lys Cys
 130 135 140
 Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg Val Glu His His
 145 150 155 160
 Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser Leu Pro Ala Arg
 165 170 175
 Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly Leu Ile Ile Leu
 180 185 190
 Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile Ile Phe Gly Val
 195 200 205
 Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn Leu Trp His Trp
 210 215 220
 Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys Glu Ser Ser Gly
 225 230 235 240
 Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly Gln Cln Gly Ala
 245 250 255
 Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys Thr Phe Pro Glu
 260 265 270
 Asp Met Cys Tyr Pro Asp Gln Gly Val Cys Gln Gly Thr Cys Val
 275 280 285
 Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg Met Leu Ser Leu
 290 295 300
 Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg Gln Met Pro Thr
 305 310 315 320
 Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr Asp Gln Leu Leu
 325 330 335
 Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro Phe Ser Glu Pro
 340 345 350
 Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys Phe Thr Gly Thr
 355 360 365
 Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr Glu Pro Leu Cys
 370 375 380

Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr Leu Gln Lys Glu
 385 390 395 400
 Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser Pro Ser Pro Asn
 405 410 415
 Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro Gly Glu Asp Cys
 420 425 430
 Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu Pro Gln Cys Ala
 435 440 445
 Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser Arg Thr Glu Ala
 450 455 460
 Arg Asp Gln Pro Glu Asp Gly Ala Asp GLY Arg Arg Leu Pro Ser Ser Ala
 465 470 475 480
 Arg Ala Gly Ala Gly Ser Ser Pro Gly Gly Gln Ser Pro Ala
 485 490 495
 Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly
 500 505 510
 Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln
 515 520 525
 Thr Ser Gln Glu Gly Ala Ala Ala Ala Glu Pro Met Gly Arg Pro
 530 535 540
 Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe Ala Gly Asn Gly
 545 550 555 560
 Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly Leu Arg Glu Pro
 565 570 575
 Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly Ala Lys Ala
 580 585 590

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1391 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (vi) IMMEDIATE SOURCE:
 - (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 - (B) CLONE: 9D-15C

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 39..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

CCGGCTGAGGC CGCGGGCCCCC CCCAGCCGTGT CCCCGCCCG ATG GCG CCG CGC GCC 53
Met Ala Pro Arg Ala
1 5
CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTC TGCG CGC CTG CTC 101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Cys Ala Leu Leu
10 15 20
GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG 149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
25 30 35
AGG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA 197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
40 45 50
AAG TAC ATG TCT TCT AAA TGC ACT ACTP ACC TCT GAC AGT GTA TGT CTC 245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Ser Asp Ser Val Cys Leu
55 60 65
CCC TGT GGC CGG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA 293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Asp Lys
70 75 80 85
TGC TTG CTG CAT AAA GTT TGT GAT ACA GCG AAG GCG CTG GTG GCC GTG 341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
90 95 100
GTC CGC GGC AAC ACC ACC ACC CCC CGG CGC TGC CGG TGC AGG GCT GGG 389
Val Ala Gly Asn Ser Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
105 110 115
TAC CAC TGG AGC CGG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC 437
Tyr His Trp Ser Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
120 125 130
GCC CGG CTG GGC CGC CAG CAC CCC TGG AAC TGC CTC AAC AAG GAC ACA 485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
135 140 145
GTT TGC AAA CCT TGC CTT GCA GGC TAC TGC TCT GAT GCC TTT TCC TCC 533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150 155 160 165
ACG GAC AAA TGC AGA CCC TGG ACC ACC TGT ACC TTC CTT GGA AAG AGA 581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
170 175 180
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT 629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
185 190 195

```

CIG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT	677
Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly	
200	205
	210
TIA ATT ATT CTG CTT CCT TTA GGG TCT GGC GGC CGT GTC GCT GCC ATC	725
Lue Ile Leu Ile Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ile Ile	
215	220
	225
ATC ATT GGC GTT TCC TAT AGG AAA AAA GGG AAA CCA CCA CTC AAC GCT ATT	773
Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu The Ala	
220	235
	240
	245
TTC TGG CAC TGG ATC ATA GAG GCT TGT GGC CGC CTA ACT GGA GAT GAG	821
Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys	
230	250
	255
	260
GNG TCC TCA GGT GAG ACT TGT GTC AGT ACA AAC ACG GCA AAC TTT GGT	869
Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr The Ala Asn Phe Gly	
235	265
	270
	275
CNG CAG GGR GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GNG GNG GAG	917
Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys	
240	280
	285
	290
ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GGT GTC TGT CAG	965
The Pro Glu Pro Asp Met Cys Tyr Pro Asp Glu Gly Val Leu Cys Gln	
295	300
	305
GCG ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GGC AGG	983
Gly Thr Cys Val Gly Gly Glu Tyr Ala Glu Gly Val Cys Ala Arg	
310	315
	320
	325
ARG CTC TCA TGT CGC AGC AMG ACC GAG ATA GAG GAA GAC AGC TTC AGC	981
Met Leu Ser Leu Val Ser Lys Thr Ile Glu Ile Glu Asp Ser Phe Arg	
315	330
	335
	340
CAG ATG CCC ACA GAA GAT GAA TAG ATC GAC AGG CCC TCC CAG CCG CCA	109
Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr	
345	350
	355
GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT OCT	157
Gln Asp Glu Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro	
360	365
	370
TTC TCT GAA CCC CTG GAG GGN GGG GAG GAG AAT GAC ATC TTA AGC CAG TGC	205
The Ser Glu Pro Leu Glu Val Gly Glu Asp Ser Leu Ser Gln Cys	
375	380
	385
TTC ACG GGG ACA CAG AGC ACA TGT GGT CTA GCA AGC TGC AAC TGC ACT	253
The Thr Gly Thr Gln Ser Thr Val Cys Ser Glu Ser Cys Asn Cys Thr	
390	395
	400
	405
GAG CCC CTG TGC AGC AGG ACT GAT TGG ACT CCC ATT AGC TCC TGT GAA AAC TAC	301
Gln Pro Leu Cys Arg Thr Pro Asp Trp Thr Pro Fer Ser Ser Glu Asn Tyr	
410	415
	420
TTC CAA AAA GAG GCG GAC AGT GGC CAG TGT CGG CAC TGG GCA GGC AGC	349
Leu Gln Lys Glu Val Asp Ser Glu His Cys Pro His Trp Ala Ala Ser	
425	430
	435

CCC AGC CCC AAC TCG GCA GAT GTC TGC ACA GGC TGC CGG AAC
 Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn
 440 445 450

391

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 451 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Pro	Leu	Phe	Ala	Leu	Leu	Leu
1										10					15
Leu	Cys	Ala	Leu	Leu	Ala	Arg	Leu	Gln	Val	Ala	Leu	Gln	Ile	Ala	Pro
	20									25					30
Pro	Cys	Thr	Ser	Glu	Lys	His	Tyr	Glu	His	Leu	Gly	Arg	Cys	Cys	Asn
	35						40					45			
Lys	Cys	Glu	Pro	Gly	Lys	Tyr	Met	Ser	Ser	Lys	Cys	Thr	Thr	Thr	Ser
	50						55				60				
Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro	Asp	Glu	Tyr	Leu	Asp	Ser	Trp
	65						70			75		80			
Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His	Lys	Val	Cys	Asp	Thr	Gly	Lys
	85						90			95					
Ala	Leu	Val	Ala	Val	Val	Ala	Gly	Asn	Ser	Thr	Thr	Pro	Arg	Arg	Cys
	100						105				110				
Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Ser	Gln	Asp	Cys	Glu	Cys	Cys	Arg
	115						120				125				
Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Leu	Gly	Ala	Gln	His	Pro	Leu	Gln
	130						135				140				
Leu	Asn	Lys	Asp	Thr	Val	Cys	Lys	Pro	Cys	Leu	Ala	Gly	Tyr	Phe	Ser
	145						150				155			160	
Asp	Ala	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Arg	Pro	Trp	Thr	Asn	Cys	Thr
	165						170				175				
Phe	Leu	Gly	Lys	Arg	Val	Glu	His	His	Gly	Thr	Glu	Lys	Ser	Asp	Ala
	180						185				190				
Val	Cys	Ser	Ser	Ser	Leu	Pro	Ala	Arg	Lys	Pro	Pro	Asn	Glu	Pro	His
	195						200				205				
Val	Tyr	Leu	Pro	Gly	Leu	Ile	Ile	Leu	Leu	Phe	Ala	Ser	Val	Ala	
	210					215				220					
Leu	Val	Ala	Ala	Ala	ile	Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly
	225						230				235				240

Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
 245 250 255
 Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
 260 265 270
 Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu
 275 280 285
 Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
 290 295 300
 Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Pro Tyr Ala Gln
 305 310 315 320
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
 325 330 335
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
 340 345 350
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
 355 360 365
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
 370 375 380
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
 385 390 395 400
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
 405 410 415
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
 420 425 430
 His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
 435 440 445
 Cys Arg Asn
 450

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3136 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (vi) IMMEDIATE SOURCE:

(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 (B) CLONE: FULL LENGTH RANK

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGGCTGAGGC CGCGGCCGCC	GGCCGGCTGTG CCGCGGCC	ATG GCC CCC CGC GGC	53
		Met Ala Pro Arg Ala	
1		1	5
CGG CGG CGC CGC CGG CTG TTC GCG CTG CTG CGC TGC GCG CTG CTC	Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Cys Ala Leu Leu	101	
15	15	20	
GCC CGG CTG CAC GTG CCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG	Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu	149	
25	30	35	
AGC CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA	Lys His Tyr Lys His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly	197	
40	45	50	
AGG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG	Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu	245	
55	60	65	
TGC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA	Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys	293	
70	75	80	85
TGC TTG CTG CAT AAA CCT TGT GAT ACA GGC AAG GGC CCT GTG GCC GTG	Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val	341	
90	95	100	
GTC GCC GGC AAC ACC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG	Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly	389	
105	110	115	
TAC CAC TGG ACC CAG GAC TGC GAG TGC TGC CGC AAC ACC GAG TGC	Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys	437	
120	125	130	
GCG CCG GGC CCT GGC CGC CAG CAC CGG TTG CAG CTC AAC AAC AAG GAC ACA	Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr	485	
135	140	145	
GTC TOC AAA CCT TGC CCT GCA GGC TAC TTC TCT GAT GGC TTT TCC TCC	Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser	533	
150	155	160	165
ACG GAC AAA TGC AGA CCC TGG ACC AAC TOT ACC TTC CCT GGA AAC AGA	Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg	581	
170	175	180	
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG CCTT TCC AGT TCT TCT	Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser	629	
185	190	195	

CTG CCA GCT AGA AAA CCA CCA AAAT GAA CCC CAT GTT TAC TTG CCC GGT
 Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly 677
 200 205 210
 TTA ATA ATT CTG CCT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC 725
 Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile
 215 220 225
 ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT 773
 Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn
 230 235 240 245
 TTG TGG CAC TGG ATC AAAT GAG GCT TGT GTG GGC CGC CTA AGT GGA GAT AAG 821
 Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys
 250 255 260
 GAG TCC TCA GGT GAC ACT TGT GTC ACT ACA CAC ACC GCA AAC TTT GGT 869
 Gln Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly
 265 270 275
 CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTC GAG GAG AAG 917
 Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Thr Leu Glu Glu Lys
 280 285 290
 GAC TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG 965
 Thr Phe Pro Gly Asp Met Cys Tyr Pro Asp Gln Gly Val Cys Gln
 295 300 305
 GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGG GAA GAT GGC AGG 1013
 Gly Thr Cys Val Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg
 310 315 320 325
 ATG CTC TCA TGT GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC AGC TTC AGA 1061
 Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg
 330 335 340
 CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA 1109
 Gln Met Pro Thr Glu Asp Gly Tyr Met Asp Arg Pro Ser Gln Pro Thr
 345 350 355
 GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT 1157
 Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro
 360 365 370
 TTC TCT GAA CCC CTG GAG GTG GGG GAG ANT GAC ACT TTA ACC CAG TGC 1205
 Phe Ser Glu Pro Leu Glu Val Gly Asn Asp Ser Leu Ser Gln Cys
 375 380 385
 TTC ACG CGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGA AAC TGC ACT 1253
 Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr
 390 395 400 405
 GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC 1301
 Glu Pro Leu Cys Arg Thr Asp Thr Pro Met Ser Ser Glu Asn Tyr
 410 415 420
 TGG CAA AAA GAG GTG GAC AGT GGC CAT TGC CGG CAC TGG GCA GCC AGC 1349
 Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser
 425 430 435

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT 1397
 Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro
 440 445 450

GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CTT GGA CCC TTG 1445
 Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Iys Arg Gly Pro Leu
 455 460 465

CCC CAG TGC GCC TAT GGC ATG GGC CCT CCC CCT GAA GAA GAA GCC AGC 1493
 Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Phe Glu Glu Ala Ser
 470 475 480 485

AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG CCT GAT GGG AGG AGC CTC 1541
 Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu
 490 495 500

CCA AGC TCA GCG AGG GCA GGT CCC GGG TCT GGA AGC TCC CCT GGT GGC 1589
 Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Ser Gly Ser Pro Gly Gly
 505 510 515

CAG TCC CCT GCA TCT GGA AAT GTC ACT GGA AAC AGT AAC TCC ACG TTC 1637
 Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe
 520 525 530

ATC TCC AGC GGG CAG GTG ATG AAC CGT ACT GGA AAC AGT AAC TCC ACG TTC 1685
 Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val
 535 540 545

TAC GTC AGC CAG ACC TCC CAG GAG GGC GGG GCG CCT GCG GAG CCC 1733
 Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Glu Pro
 550 555 560 565

ATG GGC CGC CGG GTG CAG GAG ACC CTG GCG GCG CGA GAC TCC TTC 1781
 Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe
 570 575 580

GCG GGG AAC GGC CCG CGC TTC CCG GAC CGC TGC GGC GGC CCC GAG GGG 1829
 Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly
 585 590 595

CTG CGG GAG CGG GAG AAG GCC TCG ACG CGC CGT CAG GAG CAA GGC GGG 1877
 Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Glu Gly Gly
 600 605 610

GCC AAG GCT TGAGGCCGCCCC CCATGGCTGG GAGGGCGAG CTGGGAGGCCA 1926
 Ala Lys Ala
 615

GGGCTCGOGA GGGCACCCAC GCAGGCTCTG CCCCCAGCCCC GGGCACCCAG GGATCGATCG 1986
 GTACAGCTGA GGAAGACCCAC CGGGCATCTG CTGGCCACTT TCCCTTCAG GAAATGGGCT 2046
 TTTCAGGAG TGAATTTGATG AGGACTGTCAC CCATCCCCAC GGATGCTCAG CAGCCCCCG 2106
 CACTGGGGCA GAGGTCTCC CGGCCCCACTC TCAAATTCGG AGCGATTAATG TGTGGCCACTA 2166
 TGACAGCTAT TTTCATGACT ATCCCTGTTCT GTGGGGGGGGG GGTCTATGTT TTCCCCCCAT 2226
 ATTTTGATTTC CTTTTCATAA CTTTTCTTGA TATCTTCTT CCCCCCTTTTT TAATOTAAAG 2286
 GTTTTCTCAA AAATTCCTCT AAAGGTGAGG GTCTCTTTCTT TTTCCTCTTTT CCTTTTTTTT 2346

TTCTTTTTT GCGCAACCTGG CCTCTGGCCCA GGCTAGAGTG CAGTGGTGCG ATTTATAGCC 2406
 GGTCAGCCT CTAACTCTG GCGTCAGCA ATCCAGTGA TCCCTCCCAC CTCACCTTCG 2466
 GAGTAGCTGG GATCACAGCT GCAGGCCAGG CCCAGCTTCC TCCCCCGAC TCCCCCC 2526
 CAGAGCACCA GTCCCCACCAT GTTACCCAGC CTGGGTCTCAA ACTCCCCAGC TAAAGCAGTC 2586
 CTCCAGGCTC GCGCTCCCAA ACGACTGGGA TTACAGCGCG GAGCCCCCAC CGTGGCCGTC 2646
 TTTCAGCTATT TTCTTITGCG CCCTCTCTCA CAGTGTPTTA GAGATGCGCTT TCCCAAGTG 2706
 TGTTTCTTGT AAACACTTTT GGGAAAGGGC TAAACATGTG AGGCTCTGGG ATAGTTGCTA 2766
 AGITTCGCTAGG AACACTGTGGT GGGACTTTCA TATTCGAAA ATATCTCTAT ATTCTCATTT 2826
 TTCTAAAGA AAGAAAAAGA GAAGACCGAT TTATTCCTCC TGAATCTTTT TAAGTTTGCG 2886
 TCGTTCCTTA ACCAGAACTA AGCTCACTGAT GTGACCTTAC CGGCTAGGTC GTTAATTAT 2946
 CCATGCTGGC AGAGGCACTC AGGTACTTGG TAGGAAATT TCTAAAMCTC CAAGTTGCTA 3006
 CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGAAA AGATOGAGAA AATGAACAGG 3066
 ACATGGGGCT CCGGAAAGA AAGGGCCCG AGAAGTCAAG GAAGNAATAAA GTTGAAATT 3126
 TAAAAAAA 3136

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 515 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Het Ala Pro Arg Ala Arg Arg Arg Pro Leu Phe Ala Leu Leu			
1	5	10	15
Leu Cys Ala Leu Leu Ala Arg Leu Glu Val Ala Leu Glu Ile			
20	25	30	
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys			
35	40	45	Aaa
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr			
50	55	60	
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser			
65	70	75	80
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr			
85	90	95	
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg			
100	105	110	

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
 115 120 125
 Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
 130 135 140
 Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
 145 150 155 160
 Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
 165 170 175
 Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
 180 185 190
 Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
 195 200 205
 Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
 210 215 220
 Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
 225 230 235 240
 Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
 245 250 255
 Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
 260 265 270
 Thr Ala Asn Phe Gly Gin Gin Gly Ala Cys Glu Gly Val Leu Leu Leu
 275 280 285
 Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
 290 295 300
 Gly Gly Val Cys Gin Gly Thr Cys Val Gly Gly Pro Tyr Ala Glu
 305 310 315 320
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
 325 330 335
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
 340 345 350
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
 355 360 365
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
 370 375 380
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
 385 390 395 400
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
 405 410 415
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
 420 425 430

His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
 435 440 445
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
 450 455 460
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
 465 470 475 480
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
 485 490 495
 Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ser Gly
 500 505 510
 Ser Ser Pro Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
 515 520 525
 Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
 530 535 540
 Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
 545 550 555 560
 Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
 565 570 575
 Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
 580 585 590
 Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
 595 600 605
 Gin Glu Gin Gly Gly Ala Lys Ala
 610 615

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:
- (B) CLONE: FLAG peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Tyr Lys Asp Asp Asp Asp Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 212 amino acids
- (B) TYPE: amino acid

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgG1 Fc mutain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gl Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95
Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110
Leu Pro Ala Pro Met Gin Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125
Arg Glu Pro Gin Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145 150 155 160
His Ile Ala Val Glu Trp Glu Ser Asn Gln Pro Glu Asn Asn Tyr
165 170 175
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr
180 185 190
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220
Ser Leu Ser Leu Ser Pro Gly Lys
225 230

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: CMV (R2780 Leader)

(ix) FEATURE:

(D) OTHER INFORMATION: Met1-Arg28 is the actual leader peptide; Arg29 strengthens the furin cleavage site; nucleotides encoding Thr30 and Ser31 add a SpeI site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Ala	Arg	Arg	Leu	Trp	Ile	Leu	Ser	Leu	Leu	Ala	Val	Thr	Leu	Thr
1															15
Val	Ala	Leu	Ala	Ala	Pro	Ser	Gln	Lys	Ser	Lys	Arg	Arg	Thr	Ser	
														30	
														20	

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:
(B) CLONE: RANKL

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 3..884

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CC GGC GTC CCA CAC GAG GOT CGG CTG CAC CCC GCG GCG CCT TCT GCA CCG
Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro
1 5 10 15

GCT CCG GCG CGG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC
Ala Pro Ala Pro Pro Ala Ser Arg Ser Met Phe Leu Ala Leu
20 25 30

CTG CGG CTG GGA CTG GCC CAG GTG GTC TCC AGC ATC GCT CTG TTC CTG
Leu Gly Leu Gly Leu Gly Glu Val Val Cys Ser Ile Ala Leu Phe Leu
35 40 45

TAC TTT CGA CGC CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT
Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr
50 55 60

CAC TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GAT TTG CAG
His Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Glu
65 70 75

GAC TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TCC AGG AGG
Asp Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg
80 85 90 95

ATG AAA CAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT
Met Lys Gln Ala Phe Glu Gly Ala Val Glu Lys Glu Leu Glu His Ile
100 105 110

GTG GGU CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATT GAA GGC TCA
Val Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser
115 120 125

TGG TTG GAT GTG GCC CAG CGA CGC AAC CCT GAG GGC CAG CCA TTT GCA
Trp Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala
130 135 140

CAC CTC ACC ATC ATT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC
His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val
145 150 155

ACT CTC TCC TCT TGG TAC CAC GAT CGA GGC TOG GCC AAG ATC TCT AAC
Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn
160 165 170 175

ATG ACG TTA AGC AAC GCA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT
Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr
180 185 190

TAC CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC
Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser
195 200 205

GTA CCT ACA GAC TAC TAT CTT CAG CTG ATG GGG TAT GTC GTT AAA ACC AGC
Val Pro Thr Asp Tyr Leu Glu Leu Met Val Tyr Val Val Lys Thr Ser
210 215 220

ATC AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA
Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Glu Gly Ser Thr Lys
225 230 235

AAC TGG TCG GGC AAT TCT GAA TPC CAC TTT TAT TCC ATA AAT GTT GGG 767
 Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly
 240 245 250 255
 Gly Phe Phe Lys Leu Arg Ala Gly Glu Ile Ser Ile Gln Val Ser
 260 265 270
 AAC CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT 815
 Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala
 275 280 285
 TTC AAA GTT CAG GAC ATA GAC TGAGACTCAT TTTCGTGGAAC ATTAGCATGG 914
 Phe Lys Val Gln Asp Ile Asp
 290
 ATGTCCTAGA TGTGTTGGAAA CTTCCTTTAAA AATGGATGAT GTCTATACAT GTGTAAAGACT 974
 ACTAAAGAGAC ATGGCCCCACG GTGTTATGAAA CTCAACACCC TCTCTCTTGAA GCGCTGTACAG 1034
 GTTGTGTTATA TGTAAAGTCCT ATAGGTGATC TTAGATTCAT GTGTTATTACA CAACGGTTTT 1094
 ACAATTTTGT AATGTGTTCC TAGAATTGAA CCAGATTTGG AGAGGTATTTC CGATGCTTAT 1154
 GAAAACCTTA CACGTGAGCT ATGGAAAGGGG GTCACAGTCT CTGGGTCTAA CCCCTGGACA 1214
 TGTGCCACTG AGAACCTTGA AATTAAGGGG ATGCCATGTT ATTGCAAANGA AATGATAGTG 1274
 TGAAGGGTTA AGTTCCTTTTG AATTTGTTACA TTGCCCCTGGG ACCTGCAAT AAGTTCCTTT 1334
 TTTCCTAAATGA GGAGAAAAA ATATATGTTAT TTTTATATAA TGCTTAAAGT TATATTTCAG 1394
 GTGTGATGTT TCTCTGTCAA AGTTTTGTTAA ATTATATTTC TGCTATAGTA TTGATTCAA 1454
 AATATTTAAA AATGCTCTAC TGTTGACATA TTTAATGTTT TAAATGTCAGA GATGTTTTA 1514
 ACTGGTACAC TTGTTGAAATTC CCCTGGAGGT ACTCTGAGT AAGGGGGCAG AATACTGTTT 1574
 CTGGTGACCA CATGTTAGTTT ATTTCCTTTAT TCTTTTTAAC TTAATAGAGT CTTCAAG 1630

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 294 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly	Val	Pro	His	Glu	Gly	Pro	Leu	His	Pro	Ala	Pro	Ser	Ala	Pro	Ala
	1			5				10					15		
Pro	Ala	Pro	Pro	Ala	Ala	Ser	Arg	Ser	Met	Phe	Leu	Ala	Leu	Leu	
	20			25				30							
Gly	Leu	Gly	Leu	Gly	Gln	Val	Val	Cys	Ser	Ile	Ala	Leu	Phe	Leu	Tyr
	35			40				45							

Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
50 55 60

Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln Asp
65 70 75 80

Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
85 90 95

Lys Gin Ala Phe Gln Gly Ala Val Gin Lys Glu Leu Gin His Ile Val
100 105 110

Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
115 120 125

Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
130 135 140

Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
145 150 155 160

Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
165 170 175

Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
180 185 190

Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
195 200 205

Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
210 215 220

Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
225 230 235 240

Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
245 250 255

Phe Phe Lys Leu Arg Ala Gly Glu Ile Ser Ile Gin Val Ser Asn
260 265 270

Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
275 280 285

Lys Val Gln Asp Ile Asp
290

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 954 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: huRANKL (full length)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..951

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATG CGC CGG GCC AGC AGA GAC TAC ACC AAC TAG TAC CTG CGT GCC TCG GAG	48
Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu	
1 5 10 15	
GAG ATG GGC GGC GGC CCC GGA GCC CCC CAC GAG GGC CCC CTG CAC GCC	96
Glu Met Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala	
20 25 30	
CCG CCG CCG CCTG CGG CGC CAC CAG CCC CCC GCC GCC TCC CCC TCC ATG	144
Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met	
35 40 45	
TTC GTG GCC CTC CTG GGG CTG GGG CTG GGC CAG GTT GTG TGC AGC GTC	192
Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Cys Ser Val	
50 55 60	
GCC CTG TTC TTC TAT TCA AGA GCG CAG ATG GAT CCT ATT AGA ATA TCA	240
Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser	
65 70 75 80	
GAA GAT GGC ACT CAC TCC ATT TAT AGA ATT TTG AGA CTC CAT GAA ATT	288
Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn	
85 90 95	
GCA GAT TTT CAA GAC ACA ACT CTG GAG ACT CAA GAT ACA AAA TTA ATA	336
Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile	
100 105 110	
CCT GAT TCA TGT AGG AGA ATT AAA CAG GGC TTT CAA GGA GCT GTG CAA	384
Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln	
115 120 125	
AAG GAA TTA CAA CAT ATC GTT GGA TCA CAG CAC ATT AGA GCA GAG AAA	432
Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys	
130 135 140	
GCG ATG GTG GAT GGC TCA TGG TTA GAT CTG GCC AAG AGG AGC AAG CTT	480
Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu	
145 150 155 160	
GAA GCT CAG CCT TTT GCT CAT CTC ACT ATT ATT AAT GCC ACC GAC ATC CCA	528
Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro	
165 170 175	

TCT CGT TCC CAT AAA GTG AGT CTG TCC TCT TGG TAC CAT GAT CGG GGT
 .Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
 180 185 190
 TGG GCC AAG ATC TCC AAC ATG ACT TTT AGC AAT GGA AAA CTA ATA GTT
 Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
 195 200 205
 AAT CAG GAT GGC TTT TAT TAC CTG TAT GCC AAC ATT TGC TTT CGA CAT
 Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
 210 215 220
 CAT GAA ACT TCA GGA GAC CTA GCT ACA GAG TAT CTT CAA CTA ATG GTG
 His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
 225 230 235 240
 TAC GTC ACT AAA ACC AGC ATC AAA ATC CCA AGT TCT CAT ACC CTG ATG
 Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
 245 250 255
 AAA GGA GGA AGC ACC AAG TAT TGG TCA GGG AAT TCT GAA TTC CAT TTT
 Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
 260 265 270
 TAT TCC ATA AAC GTP GGT GGA TTT TTT AGT TTA CGG TCT GGA GAG GAA
 Tyr Ser Ile Asn Val Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
 275 280 285
 ATC AGC ATC GAG GTC TCC AAC CCC TCC TTA CTG GAT CGG GAT CAG GAT
 Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
 290 295 300
 GCA ACA TAC TTT GGG GCT TTT AAA GTT CGA GAT ATA GAT TGA
 Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
 305 310 315

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 317 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu
1           5           10          15
Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala
20          25          30
Pro Pro Pro Pro Ala Pro His Glu Pro Pro Ala Ala Ser Arg Ser Met
35          40          45
Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val
50          55          60

```

Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser
 65 70 75 80
 Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn
 85 90 95
 Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile
 100 105 110
 Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln
 115 120 125
 Lys Lys Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys
 130 135 140
 Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu
 145 150 155 160
 Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro
 165 170 175
 Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
 180 185 190
 Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
 195 200 205
 Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
 210 215 220
 His Gln Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
 225 230 235 240
 Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
 245 250 255
 Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
 260 265 270
 Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gln Glu Glu
 275 280 285
 Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
 290 295 300
 Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
 305 310 315

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: cDNA

(iv) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Murine

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Murine Fetal Liver Epithelium

(B) CLONE: muRANK

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

ATG CCC CGC CGG GCC GGG CGG CGC CGG CAG CTG CCC CGG CGG CGT CTG CTG 48
Met Ala Pro Arg Ala Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
1 5 10 15

CGG CTC TGC GTG CTG CTC GTP CCA CTG CGG GNG ACT CTC CAG GTC ACT 96
Ala Leu Cys Val Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
20 25 30

CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC 144
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
35 40 45

AGC AGA TGC GAA CCA GGA ARG TAC CTG TCC TCT AAG TGC ACT CCT ACC 192
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
50 55 60

TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC 240
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65 70 75 80

TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC 288
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Glu
85 90 95

AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC AAC CAC ACG CGC CGG CGT CGC 336
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
100 105 110

TGT GCT TGC ACC GCT GGC TAC CAC TGG AAC TCA GAC TGC GNG TGC TGC 384
Cys Ala Cys Thr Ala Ala Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
115 120 125

CGC AGG AAC AAC GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG 432
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Glu His Pro Leu
130 135 140

CAG CTC AAC AAC GAG ATT ACC GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC 480
Gln Ieu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145 150 155 160

TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC 528
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
165 170 175

```

ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT 576
 Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
 180 185 190

GTC GTC TGC AGC TCT TCC ATG ACA CTG AGG AGA CCA CCC AMG GAG GCC 624
 Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
 195 200 205

CAG GCT CTG CTG CCC ACT CTC ATC GTT CTG CTC CTC TTC ATC TCT GTG 672
 Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Phe Ile Ser Val
 210 215 220

GTA GTC GCT GGT ACC ATC ATC TTC GGC GTT TAC TAC AGG AAG GGA CGC 720
 Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Arg Lys Gly Gly
 225 230 235 240

AAA CGG CTG ACA CCT ATT TTG TGG AAT TGG GTC AAT GAT GCT TGC AGT 768
 Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
 245 250 255

AGT CTA AGT GGA AAT AAG GAC TCC TCA GGG GAC COT TGT GCT GGT TCC 816
 Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
 260 265 270

CAC TCG GCA ACC TCC AGT CAG CAA GAA GNG TGT GAA GGT ATC TTA CTA 864
 His Ser Ala Thr Ser Ser Glu Gin Glu Val Cys Glu Gly Ile Leu Leu
 275 280 285

ATG ACT CGG GAG GAG AAG ATG GTT CCA GAA GAC GGT CCT GCT GGA GTC TGT 912
 Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
 290 295 300

GCG CCT GTG TGT GCG GCA GGT GGG CGC CCC TGG GCA GAA GTC AGA GAT TCT 960
 Gly Pro Val Cys Ala Ala Gly Pro Trp Ala Glu Val Arg Asp Ser
 305 310 315 320

AGG ACG TTC ACA CTG CTC AGC GAG GTT GNG AGG CAA GGA GAC CTC TCG 1008
 Arg Thr Phe Thr Leu Val Ser Glu Val Thr Glu Gly Asp Leu Ser
 325 330 335

AGC AAG ATT CCC ACA GAG GAT GAG TAC ACG GAC COG CCC TGG CAG CCT 1056
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Glu Pro
 340 345 350

TGG ACT GGT TCA CTG CTC CTA ATC CAG CAG GGA AGC AAA CCT ATA CCC 1104
 Ser Thr Gly Ser Leu Leu Ile Glu Glu Ser Lys Ser Ile Pro
 355 360 365

CCA TTC CAG GAG CCC CTG GAA GTG GGG GNG AAC GAC AGT TTA AGC CAG 1152
 Pro Phe Glu Glu Pro Leu Glu Val Glu Glu Asn Asp Ser Leu Ser Glu
 370 375 380

TGT TTC ACC GGG ACT GAA AGC ACG GTG GAT TCT GAG GGC TGT GAC TTC 1200
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Glu Cys Asp Phe
 385 390 395 400

ACT GAG CCT CCG AGC AGA ACT GAC TCT ATG CCC GTG TCC CCT GAA AAG 1248
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
 405 410 415

CAC CTG ACA AAA GAA ATA GAA GGT GAC AGT TGC CTC CCC TAC GTC GTC 1296
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val 420 425 430

AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG 1344
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly 435 440 445

CAG GAC CAT GAA CCC TTT CCA CGG TCC CTC AAA TGT GGA CCA TTG CCC 1392
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro 450 455 460

CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GGC AGC ATG 1440
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ser Met 465 470 475 480

GCA GAG CGG CGG GGA GTA CGG CCC CAG GAC AGG GCT GAT GNG AGG GGA GGC 1488
 Ala Glu Ala Glu Val Arg Pro Gin Asp Arg Ala Asp Glu Arg Gly Ala 485 490 495

TCA GGG TCC CGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT CGG AAC 1536
 Ser Gly Ser Gly Ser Ser Pro Ser Asp Gin Pro Pro Ala Ser Gly Asn 500 505 510

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG 1584
 Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gin Val Met 515 520 525

AAC TTC AAC AGT GAC ATC ATC GTG GTG TAT GTC ACC CAG ACC TCG CAG 1632
 Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Glu Thr Ser Gin 530 535 540

GAG GGC CGG CGT TCC GCA GAG CCC GAG TCG GAG CCT GTG GGC CGC CCT 1680
 Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro 545 550 555 560

GTG CAG GAG GAG ACC CTC GCA CAC AGA GAC TCC TTT GGG GGC ACC GGG 1728
 Val Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala 565 570 575

CCG CCC TTC CCC GAC GTC TGT GGC ACC GGG GCT GGG CTG CAG GAG CAG 1776
 Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Glu Leu Glu Glu 580 585 590

GGG GCA CCC CGG CAG AAG GAC GGG ACA TGG CGG CGG GTG CAG GAG CAG 1824
 Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Glu Glu Gln 595 600 605

GGT GGG CGG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA 1872
 Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Cys Ala 610 615 620

GAA TGA 1878
 Glu
 625

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 625 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ala Pro Arg Ala Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
1 5 10 15
Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
20 25 30
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
35 40 45
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
50 55 60
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65 70 75 80
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
85 90 95
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
100 105 110
Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
115 120 125
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
130 135 140
Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145 150 155 160
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
165 170 175
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
180 185 190
Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
195 200 205
Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
210 215 220
Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Arg Lys Gly Gly
225 230 235 240
Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
245 250 255
Ser Leu Ser Cys Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
260 265 270
His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
275 280 285

Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
 290 295 300
 Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
 305 310 315 320
 Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
 325 330 335
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
 340 345 350
 Ser Thr Gly Ser Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
 355 360 365
 Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
 370 375 380
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
 385 390 395 400
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
 405 410 415
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
 420 425 430
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
 435 440 445
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
 450 455 460
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
 465 470 475 480
 Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
 485 490 495
 Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
 500 505 510
 Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
 515 520 525
 Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
 530 535 540
 Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
 545 550 555 560
 Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
 565 570 575
 Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
 580 585 590
 Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
 595 600 605

Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
610 615 620
Glu
625

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15
Gly Ser Thr Gly
20

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Tyr Lys Asp Glu
5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His His His His His
5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
 1 5 10 15
 Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
 20 25 30
 Arg

Arg

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated DNA selected from the group consisting of:
 - (a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO: 10, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 139, inclusive, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 290 and amino acid 294, inclusive;
 - (b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO: 12, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 162, inclusive, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 313 and amino acid 317, inclusive;
 - (c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode biologically active RANKL; and
 - (d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c).
2. The isolated DNA of claim 1, which encodes a RANKL polypeptide that is at least about 70% identical in amino acid sequence to the native form of RANKL as set forth in SEQ ID Nos: 10 and 12.
3. The isolated DNA according to claim 1 or 2, which encodes a soluble RANKL polypeptide.
4. An isolated DNA encoding a soluble RANKL, selected from the group consisting of:
 - (a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO: 10, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 48 and amino acid 139, inclusive, and a carboxy terminus selected from the group consisting of an amino acid 290 and amino acid 294, inclusive;



COMMERCIAL MONITORING SYSTEM

- (b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO: 12, wherein the protein has an amino terminus selected from the group consisting of an amino acid 69 and amino acid 162, inclusive, and a carboxy terminus selected from the group consisting of an amino acid 313 and 5 amino acid 317, inclusive;
- (c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode biologically active RANKL; and
- (d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c).

10

5. The isolated DNA of claim 4, which further comprises a DNA encoding a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

15

6. A recombinant expression vector comprising a DNA sequence according to any one of claims 1 to 6.

16
17
18
19
20

7. A host cell transformed or transfected with an expression vector according to claim 6.

21
22
23
24

8. A process for preparing a RANKL protein, comprising culturing a host cell according to claim 7 under conditions promoting expression and recovering the RANKL.

25

9. An isolated DNA selected from the group consisting of oligonucleotides of at least about 17 nucleotides in length, oligonucleotides of at least about 25 nucleotides in length, and oligonucleotides of at least about 30 nucleotides in length, which is a fragment of the DNA of SEQ ID NO: 10 or SEQ ID NO: 12.

30

10. An isolated RANKL polypeptide selected from the group consisting of:



- (a) a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 11, wherein the polypeptide has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid between amino acid 139, Inclusive, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 290 and 294, inclusive;
- (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 13, wherein the polypeptide has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 162, inclusive, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 313 and 317, inclusive;
- (c) a RANKL polypeptide encoded by a DNA capable of hybridization to a DNA encoding the protein of (a) or (b) under stringent conditions, and which is biologically active; and
- (d) fragments of the polypeptides of (a), (b) or (c) which are biologically active.
11. The protein according to claim 10, having an amino acid sequence at least about 80% identical to SEQ ID NO: 11 or SEQ ID NO: 13.
12. The protein according to claim 10 or 11, which is soluble RANKL.
13. A soluble RANKL protein which further comprises a peptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.
14. An antibody immunoreactive with RANKL polypeptide according to claim 10.
15. The antibody according to claim 14, which is a monoclonal antibody.



16. A method of inducing maturation of dendritic cells (DC), comprising contacting CD1a + DC with an amount of a RANKL polypeptide sufficient to result in decreased levels of CD1b/c expression on the DC, under conditions promoting viability of the DC, and allowing the DC to mature.

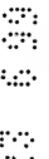
5

17. A method of enhancing allo-stimulatory capacity in dendritic cells (DC), comprising contacting CD1a + DC with an amount of a RANKL polypeptide sufficient to increase the allo-stimulatory capacity of the DC in a mixed lymphocyte reaction (MLR), under conditions promoting viability of the DC, and allowing the DC to present antigens to T cells.



10 18. A method of promoting viability of T cells in the presence of TGF β , comprising contacting T cells that have been exposed to TGF β with an amount of a RANKL polypeptide sufficient to increase the number of T cells that remain

15 viable in the presence of TGF β , under conditions that would promote viability of T cells in the absence of TGF β , and allowing the T cells to influence T cell tolerance.



20 19. An isolated DNA according to claim 1, substantially as hereinbefore described.

20 20. A recombinant expression vector according to claim 6, substantially as hereinbefore described.

25 21. A host cell according to claim 7, substantially as hereinbefore described.

22. A polypeptide according to claim 10, substantially as hereinbefore described.

30 23. An antibody according to claim 14, substantially as hereinbefore described.



C:\MYSITE\DOCUMENTS\WORKSPACES\ONE\DOC

25. A method according to claim 17, substantially as hereinbefore described.

26. A method according to claim 18, substantially as hereinbefore described.

5

DATED: 22 June 1999

PHILLIPS ORMONDE & FITZPATRICK

Attorneys for:

IMMUNEX CORPORATION

.....
.....
.....
.....
.....
.....

.....
.....
.....
.....

.....
.....



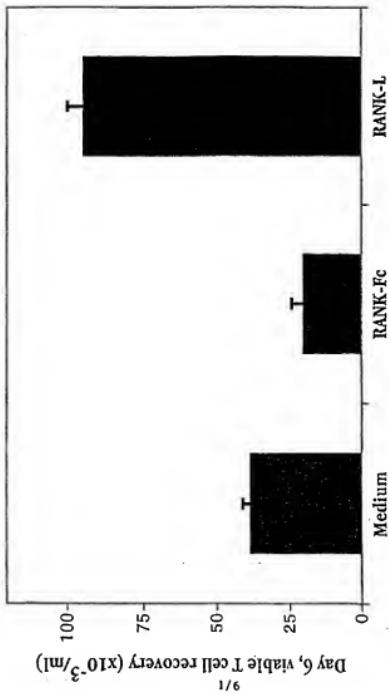


Figure 1

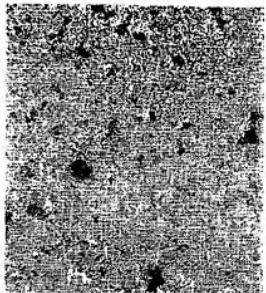


Fig. 2A

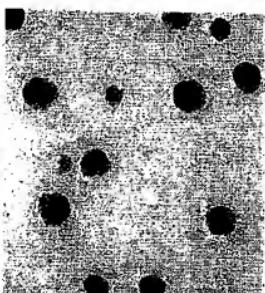


Fig. 2B

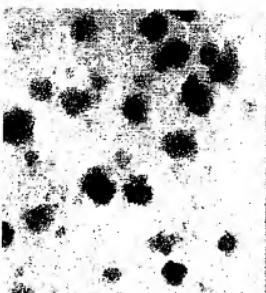


Fig. 2C



Fig. 2D

2/6

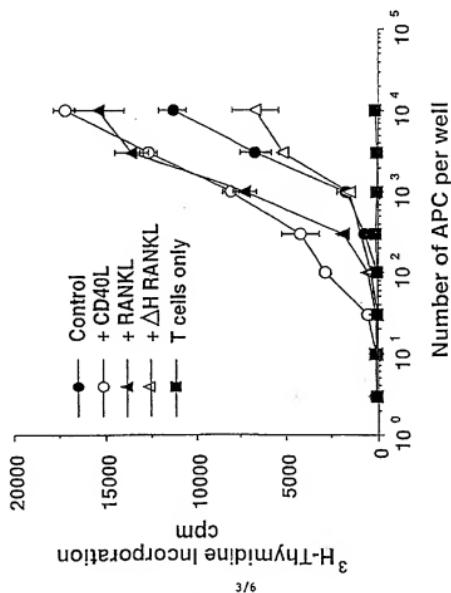


Figure 3



Figure 4

Figure 5

Hed61	KATSTHWLIL	HTVTSRIVAVSY	QTKVNLLSAI	KSPVCORETPE	GAEAK
Hed61D	KATSSPHEH	HEVQLPSASOY	PPHPVLSSQX	KNVYHSGCTSMQEF	PGLQE
Hera1	GOSTCNDLAE	HEVYVMMNSKSY	PODWENKRSQX	MHNHSGCTSMQEF	WAO
Hera1	GTNTFEDMAM	QVAVTCRPTV	PROSDEKRSQX	SHTGNGCTSMQEF	PH
Hed61	TWASSGRHAT	AVGTCRPTV	PERSLERL	THSHAKPCCQ	FG
Hed61	REASSQHAT	ASCLKSPOR	PERSLERL	PASBARNSA	SHQ
Hed61	GSGSVSEHL	LQPLRSAAAD	AALUUTDLP	KLQBLVTVC	SHQ
Hed61	...HED61	VQCINNGVOL	KLEWINKHH	KQQLVLTVC	
Hed61	PWYEDPIVY	VSCQJETKGR	USAHEINRPDV	LDPAA	QVYFG
Hed61D	PWLHGHMY	LGKQAYEFLQ	LISTTDGIVN	LVLSS	QVYFG
Hera1	RGSYGA	LTSQASQD	LYFVWXTNEHL	VNPSS	TPFG
Hera1	...LYSITYGG	LTKEAELQ	IIFVWXTNEHL	IDHD	TPFG
Hera1	...FYSINGGG	TLKSEASL	ISFWXTNEHL	JDOD	TPFG
Hed61	...QG	TLKSEASL	ISFWXTNEHL	QODA	TPFG
Hed61	...QG	VESELQG	YVFWXTNEHL	QODA	TPFG
Hed61	...QG	VESELQG	VSHGQ	...TGG	TPFG
Hed61	...QG	UWSHLSQCR	VSHGQ	...TGG	TPFG
Hed61	KHVYQNLQSF	LNDYEQNT	ISWUDTQY	ARHWHQLTQQG	AEVHG
Hed61	...QF	...QF	ISWUDTQY	IDSTTFPLEN	VLSIP
Hera1	FINAL	...QF	...QF	...QF	
Hera1	HED61	...QF	...QF	...QF	
Hera1	LYKL	...QF	...QF	...QF	
Hera1	APLVG	...QF	...QF	...QF	
Hera1	AFKVRD	...QF	...QF	...QF	
Hed61	...QF	...QF	...QF	...QF	
Hed61	...QF	...QF	...QF	...QF	
Hed61	LFRVTPEPA	QULPSPRSE	...QF	...QF	
Hed61	LYNSSD	...QF	...QF	...QF	

Figure 5 (cont.)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.